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## Test ban: new hopes, new hurdles

DISCUSSIONS on a nuclear test ban covering explosions in all environments and of all yields have dragged on for very nearly twenty years, so the optimistic noises now being made following the Geneva talks between the Soviet Union, the United States and United Kingdom should not be misinterpreted as more than a ray of hope. Even so, prospects look better now than at any time since 1963 for this major arms control measure to be brought to a conclusion—if the thorny issue of peaceful nuclear explosions can be settled.

It was in 1958 that the first international scientific discussions were held on monitoring a test ban treaty, and these, together with a growing public concern about radioactivity, led to a 1963 treaty, widely but not universally signed and ratified, which outlawed test explosions in all environments except underground. At the time it seemed that it was only modest disagreement between the United States and Soviet Union concerning on-site inspections and unmanned monitoring stations which stood between the negotiators and a truly comprehensive ban. In hindsight, however, it looks most unlikely that the United States would have been prepared to go the whole way in 1963, as ratification might have been impossible in the face of a very strong nuclear lobby.

For the first few years after 1963 a comprehensive treaty seemed out of the question politically and militarily, although seismic monitoring methods were steadily improving. Then, when seismology seemed to be incapable of offering any spectacular further developments, worries started to be made known about the possibility of evasion (by firing a shot shortly after an earthquake, for instance). And the growing vigour of the Soviet programme of peaceful nuclear explosions caused some to wonder whether military tests might be made under the cover of civil engineering. The Indian explosion in 1974 helped convert many more to this point of view.

Against this background the United States and Soviet Union made a rather odd move by agreeing on a bilateral treaty restricting tests to be below a yield of 150 kilotons. This agreement was widely regarded as a poor substitute for a comprehensive treaty (its ratification hearings are still being held in Washington) and

as a means of holding up progress on a comprehensive treaty for many years. But this was before President Carter.

It is clear that Carter attaches considerable importance to progress on a test ban. The team that the United States is fielding at Geneva is remarkably strong and contains several of those who were involved in the 1963 negotiations and who have showed continued interest in fostering arms control. For the moment, at least, the Arms Control and Disarmament Agency is in the driver's seat.

The meetings so far, in embassies, have been outside the normal Geneva disarmament forum of the United Nations Conference of the Committee on Disarmament (CCD), but if last week's optimism prevails, at some stage the matter will presumably be passed to the CCD where a more multinational analysis can be made of the technical and political issues. There are, however, still stumbling blocks. The first is that the US Congress would have to ratify the treaty, and although the nuclear weapons lobby in Washington is not what it used to be, the Energy Research and Development Administration is not likely to give away one of its birthrights without much agonising.

Second, it is by no means yet clear that the peaceful-nuclear-explosions issue can be resolved. The United States has all but abandoned its Plowshare programme and is likely to ask the Soviet Union to do the same to eliminate suspicions of misuse. The Soviet Union may find this unacceptable—a large portion of its Geneva delegation consisted of peaceful-explosions experts, which shows the importance it now attaches to these operations.

If the Soviet Union really wants to go ahead with its programme then the United States should not be allowed to get away with an excuse for stepping back from a treaty. Maybe the United Kingdom delegation should be hard at work trying to get an international agency to provide the necessary explosive. This idea has been talked about in recent years at the International Atomic Energy Agency but as yet no-one has asked them to look at the Soviet problem, which is undoubtedly a very complicated one. This is a job the United Kingdom should take upon itself. □



# What kind of food policy?

Colin Blythe and Howard Rush examine whether nutritional goals can be a basis for agricultural policy

**P**ARTICIPANTS at a number of recent conferences in Britain have tried to clarify the links between nutritional requirements, food production and supplies. Titles of meetings reflect the interest: 'People and Food Tomorrow', 'Towards a Healthier Britain' and, most recently, 'Nutritional Aspects of Food and Agricultural Policy in the United Kingdom'. All have raised a number of scientific, ethical and practical problems, and it has become apparent that until these are resolved little progress towards one coherent policy, integrating nutritional considerations, food supplies and agricultural production, seems possible. It is even argued that British policy makers remain unconvinced of the need for such a policy. What are the main arguments?

The possibility of malnutrition in some low-income groups has occasioned some concern. Clinical evidence of deficiencies is not common, however, except in certain well-defined groups, and where deficiency malnutrition exists, it is seldom due to simple poverty. Low levels of vitamin D and associated rickets occur in parts of the UK Asian community. Osteomalacia and vitamin C deficiency (not necessarily together) are found in a proportion of the elderly. Iron deficiency anaemia is said to affect about 10% of women in the UK, and folate deficiency (showing as megaloblastic anaemia of pregnancy) has occurred recently in about 2.5% of women. Deficiencies of other micronutrients may occur but the number of affected individuals is small.

In contrast, reports and papers dealing with the 'diseases of affluence' are growing more numerous; since 1970 nearly twenty reports have come from national bodies on the subject of coronary heart disease alone. As well as cardio- and cerebro-vascular disease, the list includes diverticulitis, appendicitis, constipation, certain forms of colonic cancer and, with its numerous sequelae (backache, arthritis of knees and hips, gallstones, renal failure and fallen arches), obesity.

The consensus of opinion in these reports is that some aspects of Western diet are partly responsible for the present levels of these diseases. Most conclude that wholesale changes in

diet are necessary before the major causes of illness and premature death are brought under control. The US Senate Select Committee on Nutrition and Human Needs, under Senator McGovern, has recently promulgated a set of Dietary Goals: these include a reduction of total fat intake (within which there should be a decrease in the proportion of saturated fats and an increase in the proportion of polyunsaturated fats), a reduction in dietary cholesterol, sugar and salt, and an increase in consumption of complex carbohydrates. Sweden has had a Diet and Exercise Programme, incorporating similar goals, since 1972. Norway recently announced its intention to use pricing policy as well as education in an attempt to influence eating habits.

## Evidence contested

Unfortunately, the factual evidence underlying goals of this type is contested. Disagreements over both causation and remedial measures are one reason. They have meant that the connection between diet and disease is not yet strong enough in the minds of UK policy makers for them to accord nutrition a priority in economic decisions. But having no policy amounts, in reality, to a policy of letting things drift, something which is clearly unsatisfactory. And the World Health Organisation said in 1969 that the incidence of certain diseases (coronary heart disease, for example) had reached almost "epidemic proportions".

The issues of causation may not have been resolved by clinical procedures, but then many of the tests needed to establish a causal relationship can only be performed on live human beings, and large numbers of them at that. Absolute proof may be years in coming. In the absence of absolute proof, this demands the exercise of scientific judgment based upon the best information available. Whether to wait for final proof (one way or the other), or whether to advise action now, is something which presents nutritionists with difficulties of judgment and also of conscience.

In Britain, the existing government role concerning diet is primarily one of monitoring, testing and education. In addition, a web of legislation governing food standards, additives, labelling and advertising has evolved largely on an *ad hoc* basis. Policy questions, such as whether Britain should or could feed itself, usually turn



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upon issues of security of supplies and the balance of payments. Seldom, if ever, are variations in food supplies or self-sufficiency levels related to their possible effects on the nutritional status of the population. Nor would it seem that nutritional considerations have prompted recent government interventions in food prices. Mollifying consumer unrest, assisting the economically disadvantaged and dealing with farm surpluses have been of greater concern.

The lack of nutritional reasoning behind adjustments to food supplies and prices does not, however, mean that such moves lack nutritional impact. EEC policy, for instance, is to reduce the price of certain items currently in over-supply, like butter, in order to stimulate demand, increase consumption and clear the surplus. Beyond boosting consumption artificially, this produces a secondary effect: the tendency to maintain isocaloric balance means that, as consumption of some foods rises, consumption of other items falls. In other words, by manipulating food prices for basically agricultural purposes, governments can apparently exert an effect on eating patterns and, unwittingly, on nutritional status. As recent EEC policy has sought to encourage consumption of some items which epidemiological evidence has linked with disease, the anxiety of many nutritionists to see nutritional criteria built into food policy becomes understandable.

## Basis for policy?

But if the factual evidence underlying nutritional goals is controversial, so is the advisability of using them as the basis of policy. Government intervention in dietary matters is a hotly contested issue. Among nutritionists a sizeable lobby believes that the scale of diet-related disease is such as to justify a far more radical approach to public health. This group would like to see

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pricing and educational measures employed as part of a coherent policy of trying to prevent ill health, rather than attempting to cure the results of unwise living. A shift to preventive medicine is economically attractive. Although few figures are available for Britain, recent estimates put the total costs to the US economy of heart disease and other diet-related illness at between \$30–35 billion annually. Costs to the UK economy must also be considerable. It may be that prices of alcohol, tobacco and some food items should more accurately reflect the community health costs incurred by their overconsumption.

A national programme of preventive medicine would almost certainly involve individuals assuming greater responsibility for their own health and basing their actions on adequate information. Attempts to educate the public on dietary matters, however, have a poor record of success. If changes are to occur in consumption levels public education may need to be reinforced with food price adjustments. Such procedures would have to be thought out with great care. Some evidence exists for suggesting that subsidies do not always benefit target groups as intended. So if taxes are also to be used to persuade consumers to alter their habits, steps will have to be taken to ensure the adequacy of diets among low-income groups.

Opponents of blanket government measures believe that improved screening and diagnosis are all that is required. After diagnosis, those at risk can take remedial action and those unaffected can escape being troubled or inconvenienced by wholesale measures. This view contends that, although nutritional needs can be assessed case-by-case, prescribing aver-

age requirements for entire populations on present knowledge is premature, and might even endanger some people's health. In discouraging overconsumption, it is asked, how is discrimination avoided against those who are not overconsuming, or those who, if they are at risk at all, may be at risk from under- rather than over-nutrition? And even if causation could be proved and remedial measures agreed, what of the freedom of individual choice? Is it for governments to coerce people into taking measures for their own good?

In the end, other factors may dictate the best course of action—the state of the economy, for example, or the adequacy of diagnostic facilities and personnel. Identifying every individual at risk of heart disease or stroke would involve a major programme of screening, with those placed in the 'at risk' category being examined at, say, yearly intervals, the rest perhaps every four or five years. Such a programme would add greatly to the costs of a Health Service already widely regarded as economically burdensome.

### Disagreements

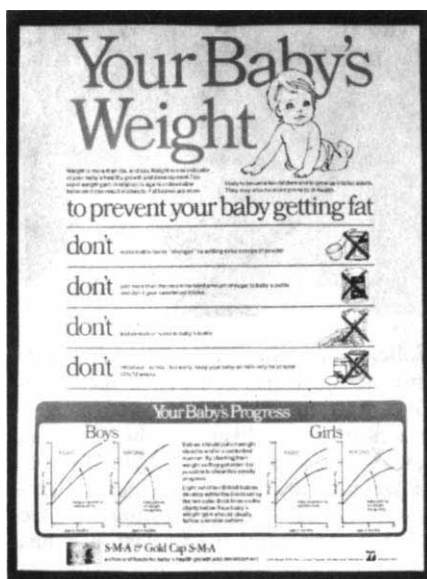
This discussion of means obscures the fact that no policy maker can be expected to act until a basis for action has been agreed. Unfortunately, there are strong disagreements over appropriate remedial measures. One example of conflicting advice to the government lies in recent evidence to the UK House of Commons Expenditure Committee considering preventive medicine. In this, the British Dietetic Association suggested that "a moderate reversal to diets in which bread and potato consumption are increased would be an advantage"; the British Medical Association felt that "the right diet for

optimal weight was relatively high in fat, high in protein and low in carbohydrates". Such advice is just as confusing to individuals who wish to follow sensible eating regimes.

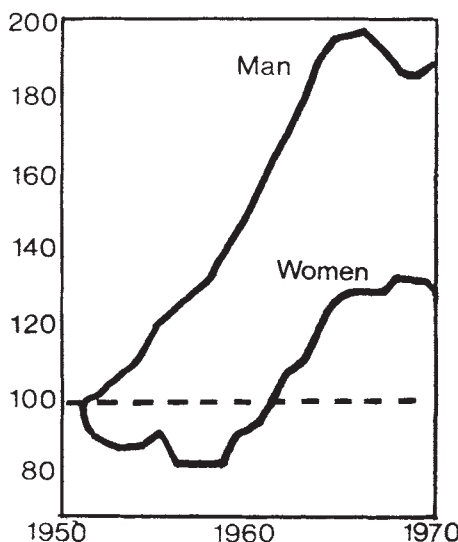
The quality and relevance as public guidelines of some other information is also open to doubt. Tools long accepted for evaluating nutritional status, such as the Recommended Daily Intake (RDI) tables, have begun to be questioned. Should the RDIs relate to a level of intake designed to secure optimum health (a concept in itself difficult to define), or to a level necessary to avoid the onset of illness? If the latter then, given that nutrient requirements vary not only for age and sex but also for bodyweight and metabolic type, illness for whom? The question of how much information now available can usefully be applied in constructing diets is also relevant. For example, is the RDI of 30 mg vitamin C per day for an adult to be secured by eating one tomato, or two? If minimum nutrient requirements cannot be assessed except on a case-by-case basis, if the notion of an 'optimal' intake is unscientific, and if the RDI tables offer no guidance on the designing of meals, what useful purpose is served by such information?

All these general problems have meant that governments have received little in the way of useful advice regarding the effect of agricultural policy on nutrition, although the fact that agricultural policy can influence nutrition is not seriously disputed. Clearly, when the major problems within nutrition are sorted out, it will be time for some cross-fertilisation to take place between disciplines. At a conference organised by the Nutrition Society in Reading recently, one example did emerge of the type of thinking that may be required to bridge the agriculture/nutrition gap.

In it, Professor W. Holmes briefly suggested some changes and problems facing UK agriculture if food goals outlined by the US Senate and others are to be realised. For example, an increase in the contribution of bread grains, vegetables and fruit to the diet, and a reduction in the intake of animal products (especially animal fats), might require a corresponding increase in the arable area and a reduction in the use of grain for animal feed. Though noting that such a change was technically feasible, he pointed out the considerable effects on the cost and profitability of farming and on the ancillary industries. A detailed study of the complex components within the farming community and of the incentives and advice necessary to achieve improvements was necessary, before any attempt to implement a food policy was likely to be successful. □



Where nutrition begins:  
Blurb for mothers and others



Graph showing percentage increase in death rates due to coronary heart disease of men and women in England and Wales in 35–44 age group



## BRITAIN

# Research on research

*The UK Social Research Council (SSRC) has approved a grant application from the Science Policy Research Unit (SPRU) of the University of Sussex to assess the criteria used by the Science Research Council (SRC) for capital projects in basic science. Chris Sherwell reports.*

It WAS perhaps inevitable. Certainly an investigation of the funding of big science in Britain had everything going for it. Hard economic times produce whipping boys, and in Britain's research community it is big science. Likewise growing disciplines generate new fields to conquer, and for the social sciences in Britain science policy is indisputably one of them. Last week's news that the SSRC is funding research on the SRC's funding of research should therefore have been no surprise; in fact the fall-out may be more than inconsequential.

No one is denying that the SSRC has the right and duty to sponsor policy research. And no one doubts the SRC is a good candidate for consideration in the field of science. It takes a major (54%) but now declining share of the £216-million science budget doled out to the research councils. Similarly, with high energy physics, astronomy, space and radio science—the traditional areas involving large capital-spending projects—consuming some 60% of that share, big science is a natural object of attention. Indeed, in annual spending terms it is comparable to research and development on the fast breeder reactor or, in its day, on Concorde.

But there are some raised eyebrows, both inside and outside the SRC. Scientists who privately frown on some of the projects sponsored by the SSRC are never too difficult to find, of course; and the SRC cannot comment officially on projects sponsored by other research councils. But some reactions last week were sceptical. The director of one big science laboratory said rather obviously that he would have preferred to see the money going to his own work. Others, however, doubt the motives behind the proposed research. There were also questions about whether the project could even be done.

The proposal, a year in gestation, is contained in a document running to 18 pages with 5 appendices. It was submitted by Dr Roy Turner and Mr Keith Pavitt of SPRU and Dr Norman Dombey, reader in theoretical physics

at Sussex, to the SSRC's political science committee. The SSRC approved the grant application in principle almost three weeks ago. The amount involved is £26,345 over two years, and the team is now looking urgently for a scientist and a social scientist so that the work can begin in October.

Their case for the study is simple. There is not as much work in Britain on the problems of science policy as there ought to be compared with North America. The SRC disposes of a large sum of money on basic research, and now faces hard choices distributing resources between big science and the rest of science. Yet, says the team, it is not known whether there is any technological benefit from, say, a high energy physics programme which could not have been achieved more cheaply by direct industrial research; nor are there guidelines on what proportions of funds should be allocated to fields in which there will be no foreseeable technological benefit.

The team says it has four aims. The first is to assess the research of six SRC centres in terms of certain criteria. The six centres are in high energy physics (CERN, Rutherford and Dares-

bury) and in astronomy (the Isaac Newton telescope, the Nuffield radio astronomy laboratories and the Mullard radio astronomy observatory). The criteria are 'intrinsic' (by which is meant the merit of the science and the researchers, and the benefits to other fields of science); 'educational' (the benefit to those who teach and those who work in other scientific fields); and 'technological' (the long term and short term application of the results and methods of research).

The team expects to spend two-thirds of its time on this aspect alone. The rest will be spent comparing the actual performance of the centres with their original justification, applying what is learned to proposals to set up new centres (the team has in mind the high powered laser facility and, though it is not in the SRC's remit, JET, the European fusion project), and finally considering British and international practice in funding big science.

In its proposal the team explains how it plans to proceed, and it is here that the difficulties may emerge. They will assess the research by speaking to scientists in the field, circulating questionnaires and examining published papers. The original justification for the centres will be

## Bondi goes to Energy

AS WIDELY expected, Sir Hermann Bondi is to replace Dr Walter Marshall as Chief Scientist in the UK Department of Energy. For the past six years Sir Hermann has been Chief Scientific Adviser at the Ministry of Defence. He goes into the Department of Energy with no strongly expressed views on the record concerning choice of reactor, the balance between nuclear, hydrocarbon and renewable energy sources, or the wisdom of a fast-breeder programme; he freely admits that he has a lot to learn.

Sir Hermann looks back on his time at the Ministry of Defence with satisfaction; in part his role has been as a person to whom anyone could turn for disinterested and friendly advice and criticism. But he has always preached mobility—sometimes to a rather hostile audience within the civil service—and he clearly practises what he preaches. In his own words, the defence post is the "fourth good job I have thrown up". The others were a lectureship and fellowship at Cambridge, a professorship at Kings

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College, London, and the directorship of the European Space Research Organisation. He admits there is a touch of masochism in this, but he felt he was getting too comfortable at Defence and was no longer an outsider. It will be interesting to see whether the Ministry of Defence has the courage to appoint another outsider.

learned, the team says, by interviewing those involved in the decisions (like Sir Alec Merrison and Sir Denys Wilkinson in the case of Rutherford and Daresbury and Sir Martin Ryle in the case of Mullard), from published sources (in the case of CERN) and if possible through access to original documents.

But the team is likely to face a critical problem over access to SRC documents. When Mike Gibbons and John Hartland did their study of CERN for the old Council of Scientific Policy (CSP)—a study the team mentions—the SRC gave special dispensation because the study was confidential to the Council; when Margaret Gowing sought SRC documents for her study of CERN on behalf of CERN, the SRC was prepared to allow access but the Department of Education and Science refused under the thirty-year rule. On that history, access is likely to be denied to the SPRU researchers.

The team does not see possible denial of access as a special difficulty. But Gibbons thinks otherwise. Not only did it take him and Hartland two years to do their study, which was of one centre, not six plus two new ones; they also found the original documents essential in providing what he calls a "well constructed net", the holes of which could be plugged with the help of interviews. Only with the documents could they appreciate just how much memories failed with time and how beneficial hindsight could be.

Whether or not the research yields controversial findings, the fact that it is going ahead at all has certainly provoked interest. The SSRC has some £25,000 at its disposal for science policy studies; this used to be in the hands of the CSP but was transferred to the SSRC when the Advisory Board for the Research Councils (ABRC) began. This award to the SPRU team means that other applications are denied. That is nothing new, except that, in going to one large project, it is ironically favouring 'big science policy' proposals at the expense of others whose merit may be as clear. More interestingly, and particularly as the research is about the SRC, the SRC is certain to have seen a copy of the proposals at an early stage and to have passed a verdict on it.

Two factors indicate why the SRC might be unhappy about it. One is the precedent of the Gowing case: lack of access is bound to reduce the value of the research. The other is its natural misgivings about the quality and partiality of science policy research, using anecdotal evidence or not. Indeed, Norman Dombey's own article on CERN, in *The Times Higher Education Supplement* earlier this year, is unlikely to have endeared this

project to the SRC. And Keith Pavitt's views on the use to which Britain's scientists and engineers are put (typically, into aircraft, nuclear reactors and weapons instead of motor cars and machinery) won't have helped.

The topicality of the subject may have assisted the SSRC in reaching a decision. But one suggestion being put about last week, which could not be confirmed, was that the SSRC's political science committee regards its support for the SPRU proposal as a test case in its efforts to establish itself as the body sponsoring research that monitors decisions in government. The project, it was suggested, is a useful addition to the pressure for a lifting of the thirty-year rule.

As far as Dr Saul Rose, the committee's chairman, is concerned, there are bound to be differing views of its

support for the project, as there are for much of the work it sponsors. As for projects that could "come up against a stone wall" because of lack of access, it was always hoped that something could be worked out; in any case projects were monitored, and could be called off or perhaps reshaped if they were unlikely to achieve the intended aims.

In the end, though, the project will be judged by its results. No one wanted to anticipate those last week, or to predict their likely impact. But one former high energy physicist privately expressed his reservations. The big science community in Britain, he said, was now highly sensitive to criticism after the financial battering of recent years. Though prepared to admit that the findings might be interesting, he couldn't see what useful purpose the project would ultimately serve. □

## Reactor choice: last lap looms

THE final and most important phase of Britain's tortuous debate over which type of thermal nuclear reactor should follow the existing Magnox generation is just beginning. Last week the Department of Energy (DEN) published extracts from the assessment made over the past nine months by the National Nuclear Corporation (NNC) of the three thermal reactor systems under scrutiny—the Pressurised Water Reactor (PWR), the Advanced Gas-Cooled Reactor (AGR) and the Steam Generating Heavy Water Reactor (SGHWR).

At the same time the Health and Safety Executive published an account from its Nuclear Installations Inspectorate (NII), responsible for nuclear safety, of its study of the generic safety issues of PWRs. And the DEN also made available a summary of the discussions on nuclear energy policy which emerged at the closed weekend seminar it held in May.

The nuclear industry, the customers, the politicians and others are expected to digest the contents of the massive NNC and NII volumes during a quiet August; discussions in September may then allow the matter to be put before the Cabinet for collective decision sometime in October. Mr Anthony Wedgwood Benn, the Secretary of State for Energy, for one, is looking forward to an interesting debate.

The NNC report says a decision has to be taken now both about the reactor systems and about actual orders "if the nuclear power option is to be maintained in a meaningful way". It says categorically that "there is no case for the adoption of the SGHWR" because there could be no operational experience of commercial sizes for a decade, there is no chance of exports and it is the most costly of the three.

Over the PWR and AGR, the report offers three options: continue with AGR, do not adopt PWR; adopt PWR, finish the current AGR programme but don't add to it; and adopt PWR, continue with AGR. The NNC says it cannot advise the first course because

it would be rejecting important export opportunities; to be grasped, moreover, these require a decision now. The second course, it says, would help exports and lead to "the cheapest available electric power for Britain", but construction of the first PWR could only start in 1980; for the course to be acceptable, ways must be found of "maintaining the confidence and enthusiasm" of those laid off by the lack of AGR work.

The third course offers the advantage of keeping open an option, but has the disadvantage of dividing available resources. "In practice", the report suggests, "it is not likely to be sensible to maintain an ordering programme for two systems beyond the early 1980s". The summary concludes that the early adoption of the PWR would have to be supported by a domestic ordering programme of more than one reactor, and that an AGR station would have to be ordered and built, if the purposes of the choice are to be achieved.

The choice of thermal reactor was also the subject of a session at the nuclear policy seminar. Although Sir Alan Cottrell, former government science adviser, disagreed, both Ned Franklin of the NNC and Walter Marshall, the DEN's former chief scientist, thought safety arguments were not a significant factor in the choice, a point borne out by the NII report, which concludes that "there is no fundamental reason for regarding safety as an obstacle" to choosing the PWR.

One interesting contribution to the seminar came from Sir Hermann Bondi, however, whose appointment to the DEN's as chief scientist was confirmed last week. It was very likely, he said, that the first major accident would be with a PWR because of their widespread adoption; there could be some advantage, he said, in being outside the main stream. As in the NNC report, the seminar summary also reveals clearly the importance attached to the matter of exports in reaching a decision.

Chris Sherwell



## BRITAIN

● British industrial journalists were almost buried under an avalanche of figures last week when four nationalised industries published their annual reports. The British Gas Corporation, the Electricity Council, the Central Electricity Generating Board (CEGB) and the Post Office all reported healthy profits. The achievements of the 'energy' industries attracted much attention. In spite of increases in the price of coal and oil and the slow increase in electricity demand, the Electricity Council and the CEGB made profits of £207 million and £130 million respectively. British Gas recorded a £31.5-million profit.

Of major concern to all three is the so-called 'energy gap' expected in the 1990s when North Sea oil and gas become scarce. Each report gives it prominence, and of course it greatly affects both short and long term research programmes. Last year British Gas spent £18.2 million on its R&D programme, the CEGB £11 million and the Electricity Council £41.7 million.

For the CEGB and Electricity Council nuclear power holds the key. But decisions over development to the year 2000 have yet to be taken, so neither has planned a detailed programme. Both, however, are improving existing reactor designs. Research is going on into the possibility of extending the life of Magnox reactors, and the CEGB is pushing ahead for the early operation of advanced gas reactors (AGRs) after the Hinkley Point Station became fully operational earlier this year. Research on the steam generating Heavy Water Reactor (SGHWR) will not increase; but the CEGB has increased its involvement in uranium exploration, and has signed agreements for joint exploration with Conwest Exploration in Canada and Urania Exploration in the USA.

Both the Electricity Council and the CEGB have faced a decline in the growth in energy demand. In view of the uncertainty this brings, the CEGB is giving priority in its more immediate research programme to improving the efficiency and safety of present plant designs rather than developing new plant with an unpredictable future. Taking into account all power stations currently under construction, it foresees no need for further new plants until 1985.

Meanwhile there is little British Gas can do to make gas supplies last into the 1990s except look for new fields and improve efficiency. Last year, as part of its energy conservation pro-

gramme, it awarded the first Gas Energy Management Award, set up to recognise better use of gas in industry. It is already supporting research to produce natural gas from coal and oil. Last year it successfully manufactured on a commercial scale a new catalyst—CRG-F—for produc-



ing substitute natural gas from oil. And at the end of March, a slugging gasifier development programme to make gas from coal, sponsored by an American consortium, came to a successful end.

This latter side of the British Gas research programme overlaps with research done by the National Coal Board (NCB). Sir Derek Ezra, NCB's Chairman, outlined the NCB's £30-million five-year programme for developing new coal technologies when opening an exhibition at the Coal Research Establishment last week. Among its priorities is the manufacture of hydrogen and substitute natural gas from coal.

Perhaps the most surprising figures were those for the Post Office. With a profit of £291.3 million, it had almost doubled the previous year's figure and had succeeded for the first time in ten years in operating its postal service profitably. Of the £51.5 million spent on R&D, the lion's share, £50 million, went on telecommunications. The biggest single project, 'System X', was supported to the tune of £12 million. This is the Post Office's comprehensive telecommunications system of the future, and if it is to be fully developed then the percentage of the Post Office's resources spent on R&D will have to increase.

● After seven weeks of intense examination, British Nuclear Fuels Ltd (BNFL) finished making its case to the Windscale public inquiry last

week. Since 14 June fifteen witnesses for BNFL have answered questions on the company's application to extend its reprocessing facilities by building an oxide fuel reprocessing plant. The key issues have been safety, the possibility of plutonium diversion and nuclear proliferation, and BNFL has by and large appeared in a good light. Now it is the turn of the Cumbria County Council and government departments to take the stand.

Just as its case finished, BNFL published its latest annual report. Although it alludes little to the inquiry, the report does mention the effect of the uncertainty surrounding future reprocessing facilities on the renegotiation of some contracts.

In his statement Sir John Hill, the company's chairman, speaks of increasing political constraints on international nuclear trade and economic difficulties in the UK and other trading nations. Still, the figures show BNFL managed to increase its sales of nuclear fuel services to £113 million last year, making a trading profit of £13.5 million, about £1 million up on the previous year. Exports contributed £11.1 million of this, down over £1 million on 1975-76. But there is little worry that the company will not have the money to carry out its investment programme: last year it successfully negotiated a loan agreement for £100 million with a consortium of British and foreign banks.

Ways have to be found of storing fuel as long as possible, especially if it cannot be reprocessed. BNFL is working in close collaboration with other countries on a vitrifying process to solidify liquid fuel for storage in water-filled ponds. The design and construction of a pilot vitrification plant is already under way, but the decision to build a production-scale plant will not be taken before BNFL's work is compared with that overseas. By the end of March, the company had set aside £13 million to pay for the vitrification in the late 1980s of all waste produced since the company was established in 1971.

Another of BNFL's activities which must expand to meet demand is the centrifuge enrichment of uranium. The first centrifuge enrichment plant started operation at Capenhurst last year and has already attracted £1,000 million-worth of business. The company also gained export orders for the conversion of enriched uranium hexafluoride to uranium dioxide using its Integrated Dry Route process.

Judy Redfearn



JET

## Delayed again

*The Council of Foreign Ministers of the European Economic Community (EEC) met in Brussels last week. Down for decision was the disputed site of the Community's fusion project, the Joint European Torus (JET). Chris Sherwell reports*

HEADS of government may make history, but in the EEC they don't appear to determine it. At their meeting in London at the end of June, they insisted that the Council of Foreign Ministers should decide the site for JET at its next meeting. In the event the Council failed to agree. And a research ministers meeting set for two days later was cancelled.

Although the UK Foreign Secretary, Dr David Owen, said last week that he was "very disappointed" by the "non-decision", British newspapers had carried well-briefed reports on the day the meeting started warning that no decision could be expected. This did not apparently contradict earlier reports of a majority in favour of Culham: the majority remained, but only belatedly was it realised that West Germany was sufficiently committed to Garching not to want the embarrassment of an immediate climb-down.

The majority itself was a change from the dark days in March when Culham, or rather Britain, fell from grace. Since then Britain has made some conciliatory gestures, notably in removing her reserve on the EEC's joint research programme, which have helped to restore the site to favour. Britain has also changed tack in her arguments, giving greater emphasis to her concern over the lack of any Community research project in the country (Germany has three) while at the same time acknowledging that Garching is scientifically as suitable a centre to take JET as Culham.

But equally the West Germans have clung tenaciously to Garching. In Britain this is seen in straight political terms: Garching is in what is called 'Strauss country', a reference to the powerful leader of the Bavarian Christian Socialist Union whose influence on potentially shifting political coalitions is so great. An alternative view, that the Germans entertain worries about a Community project going to Britain when the Labour Party is shaping up once more for internal combat over the EEC, is dismissed: it is said that the West Germans know from experience not to doubt the

commitment to the EEC of Labour's leaders. Certainly if this was an important factor, it might have been expected to have caused problems across a far broader Community front than just JET.

Not that JET is unimportant. The foreign ministers have now removed the subject almost totally from the hands of the research ministers, whose meeting last week was arranged mainly to clear up loose ends once a decision was taken. But the foreign ministers still only considered the subject informally. Their new president, Henri Simonet, himself a former EEC Energy Commissioner and now Belgium's foreign minister, spoke individually to ministers, taking his own private poll. The subject was then discussed over lunch.

No vote was taken, but it did emerge that if JET was not to go to Culham, it might not go anywhere—a possibility reportedly canvassed by the French, whose own fusion research (notably on Torus II) has been held up by the indecision over JET. Equally, however, if a vote was taken, it would apparently have gone in favour of Culham. One report had it that four countries favoured Culham, two favoured Garching, two were prepared to go with the majority, and one did not pronounce. But that accounting is probably only a 'guesstimate', at best.

Most affected is the JET team itself, based at Culham. Indeed, the lack of a decision is all the more surprising for the fact that the head of the comparable fusion project at Princeton in the United States sent a telegram ahead of the meeting to Paul Rebut, head of the JET team, and Donato Palumbo, director of the EEC fusion programme. The telegram in effect expressed horror at the possibility that JET might not go through and that Europe might not recognise its responsibilities; if JET did not go ahead, it inquired, how many of the team would consider working on the PFTR project at Princeton?

Dr Owen's appeal to the JET team to stay on at Culham, which came after the meeting, was probably made with a eye both to this and to the start of the next school year. Some of the team are thought to have already begun arranging schooling for their children in their own countries, not wishing to wait any longer for a 'final' decision. Their contracts have been extended once again, however, this time to the end of September.

By that time the Foreign ministers ought, with luck and the political will

to have decided on a site. Another meeting is set for 20 September. Between now and then Simonet is expected to visit both London and Bonn to try to iron out the difficulties. His close and long standing relationship with the present Energy and Research Commissioner, Guido Brunner, is expected to help, and though a date has yet to be set, it is on the cards that the British and German heads of government and foreign ministers will meet in Germany for one of their regular bilateral gatherings, the last of which was in London earlier this year.

All will be seeking a way to reach full agreement; a mere majority vote is inadequate under the Community scheme which allows issues not to be decided whenever any single country believes that its national interest is involved. If agreement fails yet again to materialise, JET in its existing form will almost certainly die and Britain and France will probably start initiating their own respective schemes for some other sort of project on a bilateral or trilateral basis. The British prime minister warned of this possibility after the heads of government meeting in June, and there has since been talk in similar terms in both France and Germany.

For the moment the presumption remains that they still wish to cooperate with each other, and to do so through to the Community. It is a presumption increasingly undermined by experience. □



Henri Simonet

Belgian Embassy

## EUROPE

## Nuclear reports

THE OPPOSITION which the thousands of demonstrators faced at the weekend in expressing their opposition to the Super Phénix fast breeder reactor near Lyons is not confined to the French government alone. The European Commission of the EEC last week voiced its own support for developing fast breeder technology when it released the final two parts of its three-part review on nuclear policy.

Last week's papers were on fast breeders and radioactive waste. A fortnight earlier the Commission released its first paper, on reprocessing. That report, acknowledging the Community's need for nuclear power given its desire to reduce dependence on imported energy, argued that reprocessing would help contain uranium imports and saw greater danger in not reprocessing. The Commission also proposed a special committee to consider a strategy for joint reprocessing.

Last week's breeder report was equally bullish. The limited contribution from new energy sources and the Community's limited access to uranium resources made the breeder an "essential link" in the Community's strategy. Member states had to preserve the option of making the breeder available to utilities on a commercial basis during the early 1990s.

Presenting the paper, Herr Guido Brunner, the Energy and Research Commissioner, insisted that there was no evidence to show that breeders were inherently more dangerous than existing types of reactor, although there were special security problems with plutonium.

● The OECD's Nuclear Energy Agency (NEA), in its annual report published last week, says that even under the most favourable circumstances of high availability and low demand for reprocessing services, the cumulative backlog of un-reprocessed material in member countries will increase from 4,000 tonnes U in 1977 to 16,000 tonnes U in 1986, and will remain at that level until 1990. "Uncertainties at the policy-making level" are the main obstacle to adequate reprocessing capacity, it says.

The report also says there is a pressing need to construct large scale demonstration facilities for the solidification and storage of high level waste.

Chris Sherwell

## USA

## Lost pre-eminence?

*The US Office of Technology Assessment has published a report on research in agricultural science. Colin Norman reports from Washington*

FOUR major reports in as many years have concluded that basic research in agricultural science in the United States has suffered from financial neglect, and that a modest injection of cash could pay handsome dividends. The latest, a slim volume issued last month by the Office of Technology Assessment (OTA), even goes so far as to suggest that the United States has lost its pre-eminence in basic research to increase food production, because of inflation.

The study, conducted by OTA staff with the help of two outside advisory committees, singles out three areas which should be given particular emphasis—photosynthesis, nitrogen fixation and tissue culture—and it suggests that new funding arrangements should be instituted to ensure that the cash is not siphoned off into other research.

Agricultural research in the United States is mostly funded by the Department of Agriculture, which operates its own research laboratories and channels funds to the State Agricultural Experiment Stations in the form of block grants. No funds are specifically set aside for basic research and, unlike other research agencies in the federal government it does not provide grants for university research on a competitive basis. Virtually every recent study of the agricultural research system in the United States has recommended that the department should institute a competitive grants programme, supporting projects on the basis of peer review; the OTA report is no exception.

One problem with the present system, according to the OTA study, is that since no specific funds are earmarked for basic research, a growing share of the research budget has been taken up by work on immediate problems, such as pest control and disease control, and "the net effect has been to short change basic research whose payoff is long term". Moreover, while basic research has been getting a smaller share of the pie, the pie itself has been shrinking. According to figures compiled by OTA, the Department of Agriculture's total R&D budget this year—\$570.6 million—is some \$48 million below the 1966 level in terms of purchasing power.

OTA therefore suggests (like all OTA reports, it doesn't actually come

out with a hard recommendation) that a competitive grants programme in the Department of Agriculture, specifically designed to boost funding for research on photosynthesis, nitrogen fixation and tissue culture, would pay dividends. Some \$15.6 million is now being spent in those three areas by the department, the National Science Foundation, other government agencies and private sources, the report states. It suggests that an additional \$12.5 million "appears cost beneficial" in the first year of the new grants programme, and \$4–6 million should be added each year for the next six years.

The recommendation is relatively modest. An Academy committee suggested recently that a competitive grants programme should be established with a \$60 million initial fund, rising by at least 10% a year in real terms. The programme suggested by the Academy would, however, encompass a vast range of research areas, including studies on nutrition and social policies.

There is at least some indication that repeated calls for such a programme are beginning to be heard in the federal government and in Congress. Earlier this year, President Ford recommended, as part of his valedictory budget, that \$27.6 million should be spent on a competitive grants programme in the Department of Agriculture, for a broad range of studies. The funds, in fact, were recommended by the newly-established Office of Science and Technology Policy (OSTP).

A massive agriculture policy bill, now under consideration in Congress, would also elevate research within the department's bureaucratic structure and formally establish a competitive grants programme. The bill has already cleared the Senate, and it was under debate last week in the House.

It is the appropriations committees in Congress which really count, however. Earlier this year, the House appropriations subcommittee which handles the budget for the Department of Agriculture approved only \$10 million for competitive grants in the 1978 fiscal year, \$17.6 million less than the Administration had recommended. After a bit of lobbying from the White House and from some agricultural scientists, the Senate appropriations committee approved the full \$26.7 million, however, and the conference committee which reconciled differences in the House and Senate versions last week came up with a compromise of \$15 million. □



## USA

● Following the release of a report by the National Academy of Sciences warning of potential long-term climate changes from increased burning of fossil fuels, the Energy Research and Development Administration (ERDA) last week announced the establishment of a new office to plan and conduct a research programme on the problem.

The office will have an initial budget of \$1 million, a sum added to ERDA's budget request by President Carter as part of the energy policy he unveiled last April. It will also act as the coordinating unit for other government agencies, and it will keep an eye on international research efforts.

ERDA has also established a panel, under the chairmanship of Dr Alvin Weinberg, of the Institute for Energy Analysis, to assess the potential climatic impact of increased use of fossil fuels, and to recommend a research programme. The panel is expected to produce a report in the next few weeks, and it will form the basis for the new office's research programme.

The Academy committee warned that if use of fossil fuels continues to grow at recent rates, with the industrialised countries switching from oil and gas to coal, carbon dioxide concentration in the atmosphere will probably double over the next century and increase by between 4 and 8 times over the next 200 years. The effect, the committee suggested, may be an increase in average global temperature of about 6 °C toward the end of the twenty-second century.

Though ERDA is taking such predictions seriously, as well it might since the Carter Administration's energy policy calls for sharp increases in the use of coal, ERDA officials are busy noting that there is no immediate crisis and there is ample time to switch to alternative sources of energy if the carbon dioxide problem proves to be serious.

In recent testimony before a Congressional committee, for example, Dr James Liverman, Assistant ERDA Administrator for Environment and Safety, noted that "it is clear that the scientific community cannot today offer the energy strategists a sufficiently confident estimate of the carbon dioxide impact to warrant massive changes in energy forecasting". He added, however, "we must begin discussions with the rest of the world to provide for some flexibility in long-range global energy plans, enough to permit massive reorientation, should it

prove necessary, in the next 5 or 10 years".

● Congress last week finally reached agreement on the budgets for the two key basic research agencies in the United States, the National Science Foundation (NSF) and the



National Institutes of Health (NIH). Both will get a substantial increase in funds for fiscal year 1978 (which begins on 1 October), though in the case of NSF, the boost will not be as large as the Administration had requested.

In the past few years, NSF's budget request has attracted considerable attention in Congress, as critics of the agency have sought to reduce funds for some of its programmes and to place constraints on its support of school science projects. This year, however, passage was relatively smooth, and NSF officials say they are pleased with the outcome.

NSF will receive \$861.3 million next year, a boost of \$85.4 million from this year's budget. The Administration had sought \$889 million, however, arguing that a major increase is required to offset inflationary losses incurred in support for basic research over the past decade. That argument was challenged strongly by the House Appropriations committee, which stated that "the growth of federal support of academic research has exceeded that of the gross national product by a comfortable margin and has more than compensated for the effects of inflation at a time when other important federal programs were not so favoured". The new budget will, nevertheless, keep NSF a step ahead of inflation.

The only NSF programme to feel Congress's knife strongly this year was the programme of Research

Applied to National Needs (RANN), an effort to focus research efforts on pressing national problems. RANN has long been controversial since it puts NSF firmly into the business of supporting applied, rather than basic, research. Congress decreed that RANN should get only \$63 million next year, \$15 million less than the Administration requested.

As for NIH, a compromise has been reached on the budget bill for the Department of Health, Education and Welfare (HEW), of which NIH is a part, which will significantly increase the Administration's budget request for every institute. The National Cancer Institute (NCI) will again carry off the lion's share of the increases, receiving a budget of \$867 million. The National Heart, Lung and Blood Institute comes a distant second with \$446 million. The other nine institutes will mostly receive an amount sufficient to account for inflation.

Though the amount budgeted for NIH is unlikely to be changed, the HEW budget bill nevertheless still faces some debate in both the House and Senate on provisions relating to abortion.

● President Carter last week named Arthur C. Upton, Professor of Pathology at the University of New York at Stony Brook, to be the new Director of the National Cancer Institute. The announcement, which had long been anticipated, culminates a long and intensive search for a suitable candidate.

Upton, 54 years old, is an expert on radiation injury and cancer. He recently chaired a committee which recommended termination of a controversial demonstration project, sponsored by NCI and the American Cancer Society, which involved routine breast X-rays for women under the age of 50.

The position of Director of NCI has been vacant since last September, when former Director Frank Rauscher resigned to take a \$75,000 per year job with the American Cancer Society. President Ford was reportedly set to nominate pathologist Arnold Brown of the Mayo Clinic for the job late last year, but since he was then a lame-duck president, he decided to leave the appointment to his successor. A search committee was set up earlier this year to look for suitable candidates for the post, and in April it submitted three names—Brown, Upton and Baruj Benacerraf of Harvard.

Colin Norman



## CANADA

● Atomic Energy of Canada Limited (AECL) is in trouble again. If last winter was the season of its discontent, it is hard to find a suitable description of its status this summer. Early in July, Energy Minister Alastair Gillespie announced that the Crown corporation had lost about \$130 million over a sale of a CANDU reactor to Argentina, and that he had fired the corporation's president, John Foster, as a result. Opposition members called for Gillespie's own resignation because they had warned last winter the loss would be more than \$100 million, but Gillespie had then maintained it would only be between \$23 million and \$40 million.

Gillespie rejected their criticism. He did so on the ground that he had invited Ross Campbell to join the board of directors of the corporation, to act as chairman of AECL, and to renegotiate the AECL contract with Argentina. Campbell was a former ambassador to Japan. Gillespie told the House of Commons earlier this year that the renegotiation would reduce the losses, estimated by an auditor-general's report to amount to \$100 million because of inflation, by about \$60 million to \$80 million. The renegotiation was to be made on the basis of the sale of a second reactor.

But Campbell's appointment as acting president of AECL temporarily to replace John Foster brought more criticism. The New Democratic Party's energy critic, T. C. Douglas, asked incredulously in the House: "Do I understand the minister is telling me that prior to the signing of a renegotiated agreement he was given the information that the loss would be cut to somewhere between \$23 million and \$40 million, that he now finds that those who drafted the agreement and signed it have incurred a loss of \$130 million, and that the man who carried on the negotiations now is being appointed president of AECL?"

Gillespie should resign, said Douglas. So did Allan Lawrence, of the Progressive Conservative Party. But Gillespie defended the earlier figures on the loss, saying it was the best information the company had at the time. He defended Campbell by saying that a team of negotiators was involved in the Argentina talks, and that Foster, not Campbell, was then the corporation's chief executive officer.

The government has repeatedly found itself in difficulty over the Argentina sale. At first it was opposed on the ground that it could lead to

nuclear weapons proliferation, because Argentina was regarded as unstable politically. Then the House of Commons' Public Accounts Committee questioned a \$2.4 million commission paid by Canada for AECL's sale of the reactor to Argentina. The commission was paid into a Swiss bank account held by a trading firm in Liechtenstein, but AECL did not know the name of the agent or whether it was the Liechtenstein firm.

● Another summer landmark has been the passing by the House of Commons of the bill re-organising the federal fund-granting agencies for university research, Bill C-26. Changes in the granting councils were recommended years ago by the Lamontagne committee, the Senate special committee on science policy. The government promised them as long ago as 1974.

Under the new legislation two new fund-granting councils will be created—the Natural Sciences and Engineering Research Council and the Social Science and Humanities Research Council. The former will take over the granting function of the National Research Council, leaving it free to concentrate on its other functions. The latter will take over responsibility for awards in the social sciences and humanities, and the Canada Council, which formerly handled such grants, will deal solely with the arts.

A new Inter-Council Coordinating Committee will maintain liaison and communications between the three award-granting bodies (the third being the Medical Research Council, whose function remains largely unchanged). The new committee, made up of the three council presidents, will be chaired by the secretary of the Ministry of State for Science and Technology.

In discussion in the House, the Minister of State for Science and Technology, Mr Hugh Faulkner, said he believed the re-organisation of the granting councils "will open the way to a new relationship between the government and universities in research matters". He noted that, in the past, there has been "little, if any, coordination of university research objectives regionally or nationally."

Curiosity-oriented research must continue to receive support, said Mr Faulkner, but a more selective process is needed to allow the relatively few individuals whose research is of recognised quality to be supported well—perhaps better than in

the past.

The *laissez faire* attitude to the support of university research had a much narrower validity than it once had. This meant the time had come for a "more activist" approach by the granting councils.

● The chairman of the Science Council of Canada, Josef Kates, used the publication of the council's eleventh annual report as an occasion to punch home a theme that has become perhaps the organisation's most important: technological sovereignty.

In his annual statement in the report, Dr Kates said that if Canada was to create long-term stability and respond to the dictates of Canadian geography in an increasingly interdependent world, it must stimulate and control the development of technologies essential to the well-being of the nation. "In this last quarter of the twentieth century, Canada will need to assert *technological sovereignty, that is, to develop and control the technological capability to support national sovereignty*," he said (his emphasis).

Because of historical factors and recent policies, Canada found itself in a weak position industrially, Dr Kates said. Industries that had been the leading edges of the industrialised economies for the last twenty years were the weakest in Canada. To attain technological sovereignty, Canadians would have to control technologies considered vital to Canada; develop indigenous technological capabilities in selected areas (with government adopting a "buy Canada" policy); encourage internationally-competitive high technology firms in specific areas and develop small, innovative technical firms; and set up major government-sponsored programmes to meet national needs.

At a press conference releasing the report, John Shepherd, executive director of the council, said the first step required in getting major programmes into operation (long a council objective), was to get Cabinet agreement. He referred to the history of oceans systems as a classic example of what happens to major programmes in Canada: Council-identified ocean systems as a priority area in 1971, and Madame Jeanne Sauvé, then Minister of State for Science and Technology, approved the idea in a policy statement in 1973. But to date, the government has set no priorities for the programme.

David Spurgeon

## IN BRIEF

**Séveso row**

When the release of dioxin at Séveso last year was considered at an ecotoxicology workshop held this week under the auspices of NATO's Ecosciences panel, the means of assessing the extent of dioxin poisoning in humans generated heated debate. Dr Guiseppe Reggiani, director of medical research for Hoffmann-La Roche, claimed that chloracne, the disfiguring skin disease observed in some 100 Séveso schoolchildren, was an "obligate sign of dioxin contamination".

Professor Silvio Garattini and Dr Gianni Tognoni of the Mario Negri Institute, Milan, the two main Italian participants, delivered scathing replies. Garattini described Reggiani's statement as "dangerous", and insisted that there was "no acceptable scientific evidence" to support it. He couldn't accept the implication that

the surveillance programme of all residents, and not just children, was a waste of time. Reggiani adhered to his view, and for the future forecast that there would be "no severe health damage to the population at Séveso". The Italians remained unconvinced.

**Lead in water: report**

The UK Department of the Environment last week published detailed results of a survey carried out in 1975 on levels of lead in Britain's drinking water. The main findings showed that approximately 4% of houses in Britain received water containing more than 0.1 mg of lead per litre during the daytime. About 9% exceeded this level first thing in the morning after water had been standing in pipes during the night. Less than 1% regularly exceeded 0.3 mg per litre.

The survey found that water fed into the public supply in Britain con-

tains minimal levels of lead; but in some areas it has a tendency to dissolve lead in pipes. In a statement, the minister, Mr Peter Shore, said there was a need for more research into the precise causes of lead in water, possible ways of minimising it and the effect it has on health.

The World Health Organisation's upper limit for lead concentrations in water is 0.3 mg per litre.

**Oil spill policy**

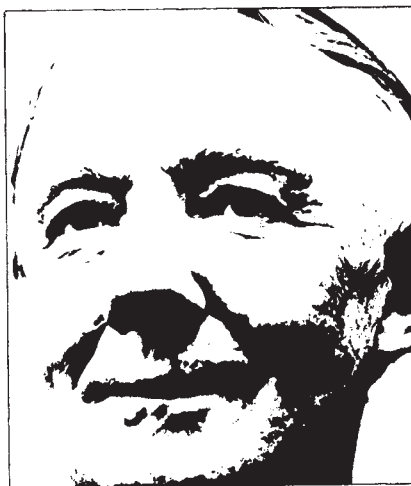
The government set out its policy for coping with oil spills at sea last week. Wherever possible, it states, oil spills should be left to degrade naturally; but where there is a serious pollution threat chemical oil dispersants should be used. Provision will be made to cope with spills of 16,000 tonnes per day offshore and 1,000 tonnes per day onshore and in inshore waters.

FOR the last ten years we have been bombarded with propaganda about impending world overpopulation. Even in countries like Britain, where the increase is relatively slow, it has been pointed out that if this continues we too will have 'standing room only' within a few hundred years. Though this will not happen, things could become more uncomfortable. But planning even for shorter periods such as the next twenty or thirty years could be much more effective if we had reasonably accurate population forecasts. Our Office of Population Censuses and Surveys (OPCS) makes a valiant effort, but their results have, so far, not been very encouraging.

During the first twenty years after the 1939-45 War, the birth rate was unexpectedly high, particularly among the middle classes, when Britain's 'top people' proudly announced in the *Times* newspaper that they had produced "Jemima, a sister for Jason, Emma, Susan, Giles, Jeremy and Natasha". The country had a minor population explosion. Then during the last ten years the forecasts have been bedevilled by the unexpected and catastrophic drop in the birthrate, so that Britain's population is actually falling.

The effects of this recent change are well illustrated in the recent OPCS Monitor giving population projections for England and Wales up to the year 2010. In 1970 there were about 800,000 live births; the estimate for the total population for 2010 was 62 million. By 1976 births had fallen by some 200,000, and based on this figure the forecast

for 2010 was only 51 million. It will thus be seen that, using the methods now available, very small changes in the birthrate have substantial effects on further population projections. Every baby not born this year will subtract

**People projections****KENNETH MELLANBY**

something like 50 individuals from the figure for 2010.

In less developed countries, population increase results mainly from the falling death rate. This is less important in Britain, where people mostly live longer, but the OPCS suggest that over the next 40 years there may be a further fall of 10% in the death rate, mainly affecting the young (infant

and neonatal mortality) and the aged. This would produce a larger number of men and women over 70, and impose an increasing burden on health services and pension funds. But will this forecast be any more accurate than that about births? We know that rats underfed in their youth live much longer than their littermates who receive a generous diet. Are overfed babies, toddlers, teenagers and adults likely to survive as well as previous, more austere raised, generations?

When Britain's population was growing rapidly, in the late 1960s and early 1970s, there were many calls to our various governments to implement population policies to produce, as soon as possible, stability ('zero population growth'). Both Labour and Conservative governments made sympathetic noises, they set up commissions and committees, and they did absolutely nothing.

Britain's politicians must now be kicking themselves for not being able to take the credit for the greatly reduced birthrate. If only in 1969 or 1972 they had promulgated a definite policy—any sort of policy—and had appeared to act vigorously, they could now point out that although they had not stimulated economic growth, built more houses or cured unemployment, their population policy had been completely successful. However, the cynic may say that, had government encouraged population stability, we would all have responded by breeding like rabbits.



# correspondence

## Translating Chinese texts

SIR,—Whilst preparing a written translation of taxonomic material from Chinese into English some time ago, I was struck by the great disparity between the highly specialised vocabulary of the subject and the simple nature of the grammar employed. This led me to wonder whether Chinese character data-handling techniques, with which I was then involved, might be applied to taxonomic texts to transform them relatively directly by machine into pidgin-English, adequate to convey the original data to the English-speaking reader, working in the appropriate scientific field. This approach seemed viable because Chinese in its written form is morphemic, each character conveying a quantum of meaning, and pidgin, which is itself primarily a direct transformation of Chinese sentences into English on a word-for-word basis, is a basic means of communication over parts of the Pacific Ocean.

An early stage of experiment, using a flowchart which was followed manually, showed that the original idea was promising, but the time-consuming nature of the work meant that relatively little material was processed. Later experiment, however, has involved actual machine processing of a number of texts, and the original ideas have been refined to accommodate some idiosyncrasies of Chinese word order, and to allow smoothing of the finished product. However, these improvements have been achieved without the need to use manual pre- or post-editing, the aim always having been to produce intelligible output by a routine operation which can be run by an operator whose only skill need be the identification of the Chinese

characters in the original text.

The process which has been developed is not a linguistically-based general translation programme. It relies for its effectiveness on the careful selection of multi-purpose glosses to represent multi-functional Chinese units in such a way that the reader's general knowledge of the field forces him into the correct choice of gloss for the context facing him. It is only in restricted areas, such as that found in taxonomic texts, that successful transformation can operate, and then it will only produce pidgin, however smoothed, rather than everyday English. However, I feel that the results so far indicate probable eventual success in providing a workable first sieve for the assessment of the large bulk of Chinese taxonomic data which at present seems to be largely unavailable to the Western scientist.

In spite of its inherent interest, it would be of little practical use to develop pidgin transformation further in the absence of any live demand for such a facility. If any readers feel that this type of transformation of taxonomic material would be of benefit to them, I would be grateful to hear from them. To give some idea of the form of output from the process, I append the result of one machine transformation, which shows the general style, and also illustrates how the multi-choice glosses operate (see below).

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## Wind power for the UK

SIR,—Some comments are necessary on Martin Ryle's article on 'Economics of alternative energy sources', (12 May,

page 111). Whilst his suggestions on storage systems may be useful, his claim that wind power could be a substantial energy resource without much environmental impact is grossly exaggerated.

The coastal area of the UK is not  $1.5 \times 10^5 \text{ km}^2$  as stated in the article; in fact the total land area of the UK<sup>1</sup> is only  $2.4 \times 10^5 \text{ km}^2$ . To obtain an annual output of  $0.56 \times 10^9 \text{ GJ}$  at a mean density of  $2.8 \text{ GJ m}^{-2}$  from a swept area that is  $4 \times 10^{-3}$  of the ground area (all figures quoted), one would need an area of  $5 \times 10^4 \text{ km}^2$ . Thus a fifth of the total area of the UK would have to be covered with towers about 60 m in height at distances apart of about 1 km. It is questionable whether this is practicable. The costs of siting the aerogenerators over all types of country have not been estimated.

In any case the visual impact on the environment would be overwhelming, and in our windier more scenic areas the view would mainly consist of endless rows of towers stretching in every direction. Also the question of noise from the 1 MW turbines, which will be only 1 km apart, is not mentioned.

A more practical upper limit on energy from wind power has been estimated by the Energy Technology Support Unit at Harwell to be the equivalent of about 8 million tons of coal a year from  $10^4$  generators each of about 1 MW rating<sup>2</sup>. Without an immense cost to the environment wind power could not make a substantial contribution to Britain's energy requirement.

C. F. CLEMENT

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<sup>1</sup> The New Oxford Atlas (Oxford University Press, 1975).

<sup>2</sup> Energy R&D in the United Kingdom. A Discussion Document (1976).

1 Trinucleus Murchison 1839.

2 glabella(e/r) all/both(have) convex {front} lobe(s), (has/have)  
3 three pair(s) short, deep glabella(e/r) lateral furrow(s), rear two  
4 pair(s) relatively deep relatively long;

5 fringe(s) upper lamella(e)'s small pit(s), irrespective of whether at/on  
6 anterior portion and/or lateral portion(s) equally deep, distributed  
7 within the radial sulcus/l.

8 pygidium/al broad, breadth approximately is length's 3 cr 4 times.

9 genotype: Trinucleus fimbriatus Murchison.

10 Period distributed: Asia and(also) Europe  
11 and(also) middle Ordovician.

early

# news and views

## Experimenting with controlled gravity

from P. C. W. Davies

OUR everyday experience of gravity is so pronounced that it is easy to forget what an incredibly weak force it really is. Only our proximity to a mass as large as the Earth enables terrestrial gravitational forces to compete with other forces of nature. By contrast, in the laboratory, gravity is almost completely undetectable: the force of attraction between two eggs in contact is a puny  $10^{-6}$  dyne. This state of affairs has led to an extreme paucity of actual experiments in gravitational physics, because most of them have to rely on the arrangement of astronomical masses in the Solar System to produce effects which are sufficiently strong to be detectable. Unfortunately, the limited repertoire of planetary activity prevents some features of gravity from being measured at all. There have been two major theories of gravity: Newton's and Einstein's. Both have relied heavily on astronomical effects in the Solar System for their verification.

Because gravity is so ill-investigated, almost any proposal for a new experiment is welcome. In a paper in this month's *Physical Review*, three physicists, V. B. Braginsky of the Moscow State University, C. M. Caves and K. S. Thorne of Caltech (*Phys. Rev. D*, **15**, 2047; 1977), propose a collection of novel experimental techniques for measuring gravitational effects, some of which have never been observed but are predicted by theory. The essence of the new proposals is that rapid advances in technology are opening up the prospect of detecting in the laboratory sources of gravity which are under the control of the experimenter. None of the experiments discussed is currently feasible, but several could become so within a decade with sufficient research and development. Some of the technology gained could even have useful spin-off in other fields.

Laboratory-generated gravitational

forces are so tiny that equipment of immense sensitivity is needed to detect it. Three new low-dissipation sensing systems are considered by the authors: a torque balance made of quartz or sapphire fibres cooled to 0.1 K, massive dielectric monocrystals cooled to millidegrees, and microwave resonators with superconducting walls. Typically, the source of the fields would be a mass of about 100 kg rotating about 1,000 times a second, which would be capable of producing accelerations due to higher order (post-Newtonian) effects in its vicinity of about  $10^{-17}$  cm s $^{-2}$  (or  $10^{-20}$  g). The major technological problem is how to isolate this effect of interest from other environmental disturbances, particularly seismic effects, which are many orders of magnitude larger. One answer is to conduct the experiments in Earth-orbiting satellites; less expensively, some of them could be performed in tandem and the seismic perturbations subtracted, or they could utilise high frequency time-dependent fields, far above natural seismic frequencies.

Some of the experiments are reminiscent of nineteenth century electrodynamics. One involves the analogue of Ampère's experiment, which demonstrated magnetic forces between current-carrying wires, only in this case the role of the electric current is taken by rotating masses one of which is part of a torsion balance. The rotation period of the other mass is modulated at the natural frequency of the balance in an attempt to set up torsional deflections. A corotating arrangement causes repulsion, and counter-rotation attraction. This 'magnetic' gravity force is expected on theoretical grounds, but has never been observed. Even in ideal laboratory conditions, using light but tough ceramic masses, the problems of untangling the minute 'magnetic' effects from the already tiny, but much larger Newtonian gravitational attraction, not to mention the disturbances from seismic vibrations, are daunting. Nevertheless, the authors do not think it

beyond the ingenuity and technological expertise of the next 'generation' of experimenters.

Other possible experiments using the torque-balance are a check on any time-dependence in the strength of gravity (which is predicted by some theories) and a measurement of the gravity produced by magnetic stresses. In the former set-up the entire apparatus, consisting of two massive balls straddling the balance, is made out of monocrystal sapphire cooled to 2 K and kept in a constant temperature environment to eliminate thermal fluctuation noise. After one month, a limit on variation of the Newtonian constant  $G$  of about one part in  $10^{11}$  yr $^{-1}$  could be achieved, which is already comparable to astronomical techniques. Turning to the latter experiment, it is particularly important for theory, because general relativity predicts that not only mass, but stress, contributes to gravity. Indeed, in some astrophysical situations, stress-induced gravitational fields can be extremely important, so a direct measurement of the gravity of stress would be of great significance.

In another class of experiment the authors discuss the use of a toroidal cavity filled with a standing-wave pattern of electromagnetic radiation. If this resonator is placed at rest round a rotating mass, according to the theory of relativity the wave pattern should be dragged around in the direction of rotation, an effect known as the dragging of inertial frames. For a mass of 5,000 kg rotating with an equatorial speed of 1 km s $^{-1}$ , the dragging effect works out at a mere  $6 \times 10^{-21}$  radian s $^{-1}$ , which is small by any account. Nevertheless if future technology enables the construction of a resonator having superconducting reflecting walls with a damping time (against decay of the wave pattern) of 10 $^5$  s—which is difficult but not outrageous—then deflections in the wave pattern of  $3 \times 10^{-16}$  radians are feasible, which might be detectable over a duration of about 2 weeks. An improvement in the



reflection coefficient  $R$  of the cavity walls, from the presently attainable  $1-10^{-11}$ , to  $1-10^{-14}$  would be necessary to sustain an experiment of this duration.

The third class of experiment utilises massive dielectric monocrystals in which the experimental arrangement is such as to eliminate the ordinary Newtonian force, which usually dominates all else, and to detect just the post-Newtonian subtle correction effects of interest. One of these experiments is the analogue of the Faraday effect in electrodynamics, in which a changing magnetic field threading a detector coil induces an electromotive force (the principle of the transformer). In the gravitational variant, the 'magnetic' induction is produced by a uniformly rotating vertical cylinder set coaxially with an axisymmetric, specially shaped, sapphire crystal, which is free to perform torsional oscillations. As the cylinder is moved up and down at the natural frequency of these oscillations, changing 'gravimagnetic' flux linking the two masses drives the torsional oscillations. The force involved is incredibly small—producing accelerations of only  $10^{-18} \text{ cm s}^{-2}$ . In this regime even the act of measurement of the oscillations could introduce Newtonian gravitational forces between the masses which

are of comparable strength.

Less feasible experiments are also discussed in which gravitational fields are detected which originate, at one extreme with the uneven distribution of matter in the Galaxy, and at the other with a beam of relativistic protons from a storage ring. In the latter experiment, a pulse of high velocity protons passes close to a monocrystal detector at periodic intervals in an attempt to set up resonant oscillations through gravitational coupling. If successful, the experiment would discriminate between scalar, vector and tensor gravitational fields, and is hence of considerable theoretical interest. Technological problems abound though, and little investigation of disturbing effects, such as bombardment of the detector by radiation from the storage ring, has yet been conducted.

All in all, this analysis provides a speculative but thought-provoking basis for more detailed feasibility studies. Much of the current impetus in experimental gravity research is behind astronomical and spaceflight technology. With the colossal cost involved in conducting extraterrestrial experiments, perhaps the time has come for a more penetrating appraisal of the possibilities for detecting and measuring laboratory-manipulated gravity. □

is a polyprotein which is cleaved to form various non-structural polypeptides, probably including components of the viral replicase (Brzeski & Kennedy *J. Virol.* **23**, 420; 1977). It seems that a similar type of translational control is employed during the replication of some plant viruses. Tobacco mosaic virus RNA, for example, directs the synthesis *in vitro* of two non-structural polypeptides which are components of the viral replicase, but the coat protein gene located near the 3' end is not translated. The functional mRNA for coat protein is a subgenomic species found in infected cells (Hunter *et al. Nature* **260**, 759; 1976).

Currently, interest in the translation of the RNA tumour virus genome is at fever pitch. These viruses replicate by transcription of a DNA provirus integrated into the host cell genome, and virus-specific polysomal RNA is of the same sense as the genome RNA. The reason for the current excitement is the clear demonstration from several groups that the genome RNA of viruses which induce sarcomagenic cell transformation (non-defective viruses) contain a distinct gene (*src*) located near the 3' end (Joho *et al. Proc. natn. Acad. Sci. U.S.A.* **72**, 4772; 1975; Wang *et al. J. Virol.* **16**, 1051; 1975; Stehelin *et al. J. molec. Biol.* **101**, 349; 1976). Identification of the *src* gene product is crucial to our understanding of the mechanism of cell transformation. Since transformation-defective viruses lack this gene, comparison of the *in vitro* translation products of their RNA with non-defective virus RNA should yield information on the nature of the *src* polypeptide.

The genome of RNA tumour viruses consists of two identical 35S subunits each of molecular weight  $3.3 \times 10^6$  and containing genes for internal structural proteins (*gs* antigens, *gag* gene) envelope glycoproteins (*env* gene) and the virus RNA-dependent DNA polymerase (*pol* gene) in addition to *src*. The genes of avian oncornaviruses have been mapped in the order 5'-*gag-pol-env-src*-3' (Wang *et al. Proc. natn. Acad. Sci. U.S.A.*, **73**, 1073, 1976), and it is highly probable that the same gene order occurs in the murine oncornavirus genome.

Recently Alan Smith's group at Imperial Cancer Research Fund translated the genome RNA of Rous sarcoma virus in a variety of cell-free protein-synthesising systems (Pawson *et al. J. Virol.* **19**, 950; 1976). In each case the product turned out to be a polypeptide of molecular weight 76,000 which was related to the internal structural proteins (*gag* gene) as judged by immunoprecipitation and peptide mapping. The other virus gene products

## Reading the tumour virus genome

from B. W. J. Mahy

THE strategem by which an RNA virus directs the host cell to synthesise virus-specific protein components continues to fascinate biochemists. Broadly, the RNA viruses are comprised of two groups, those in which the genome RNA acts as messenger for protein synthesis (positive-strand viruses) and those in which the genome RNA is of the opposite sense to messenger, and must be transcribed into a complementary RNA species (cRNA) to initiate multiplication (negative-strand viruses). The RNA tumour viruses (oncornaviruses) along with picornaviruses (such as polio), togaviruses (such as Sindbis) and most plant viruses, fall into the first group. Thus genome RNA extracted from these viruses is an active messenger in cell-free protein synthesising systems and, in the case of picornaviruses, RNA of genome size is found on polysomes of infected cells where it translates into a full-length

polyprotein that is subsequently cleaved into the capsid and other virus-specific proteins.

But in many other cases the situation is not so simple. The 42S genome RNA of togaviruses is infectious and can direct the synthesis of some polypeptides in a cell-free system but these are not the virus structural polypeptides. A subgenomic species of RNA (26S) found on infected cell polysomes is the messenger for the synthesis of a polyprotein which undergoes post-translational cleavage to form the virus structural proteins (Cancedda *et al. J. Virol.* **14**, 652; 1974; Clegg & Kennedy *FEBS Lett.* **42**, 327; 1974; Simmons & Strauss *J. molec. Biol.* **86**, 397; 1974; Wengler *et al. Virology* **61**, 120; 1974). This 26S RNA represents one-third of the genome and is located inward from the 3' end of the 42S RNA (Kennedy *J. molec. Biol.* **108**, 491; 1976). Translation of the 42S RNA itself is initiated at a single site near the 5' end (Cancedda *et al. Cell* **6**, 215; 1975) but terminates before the structural protein genes; the product

were not detected, and no differences were observed between non-defective and transformation-defective virus RNA. Duesberg's group obtained similar results (von der Helm & Duesberg *Proc. natn. Acad. Sci. U.S.A.* **72**, 614; 1975).

An important finding by Pawson *et al.* was that the 76,000 molecular weight product could be labelled *in vitro* with chemically formylated  $^{35}\text{S}$ -Met-tRNA $^{\text{Met}}$ , which only labels at the N-terminal position. This confirmed that the 76,000 polypeptide was a primary translation product and not derived from cleavage of a larger molecule.

In this issue of *Nature* (page 416) Pawson *et al.* have extended their work by examining the translation products of RNA obtained from Rous sarcoma virus-infected cells. Two size classes of virus-specific RNA, one around  $3 \times 10^6$  daltons (35S) as in the viron, the other around  $1.3 \times 10^6$  daltons (24S), are found in the cytoplasm of cells infected with avian sarcoma virus (Bishop *et al.* in *Animal Virology*, ed. Baltimore D. & Huang A. S., Academic Press, 1976). Pawson *et al.* find that the intracellular 35S RNA when added to cell-free protein-synthesising systems codes for only one polypeptide of molecular weight 76,000 which corresponds exactly to the *gag* gene product synthesised from virion RNA. However when they add to the system 20–25S RNA from infected cells, a new polypeptide of molecular weight 70,000 is synthesised, and this is immunoprecipitated only by antiserum against the virus glycoproteins. It seems therefore that the 20–25S from infected cells encodes the *env* gene and is a subgenomic species similar to the 26S RNA of togaviruses.

Results in agreement with these have been obtained by Hanafusa's group at the Rockefeller University, who micro-injected either 35S or 21S RNA from avian leukemia virus-infected cells into chick embryo fibroblast cells infected with the Bryan high-titre strain of Rous sarcoma virus, which lacks the ability to synthesise its own envelope glycoproteins. Production of infectious Rous sarcoma virus provided a sensitive assay for envelope mRNA and it was found that 21S RNA but not 35S RNA expressed this activity (Stacey *et al.* *Proc. natn. Acad. Sci. U.S.A.* **74**, 1614; 1977).

Recently, very similar findings have been made with the murine RNA tumour viruses. Both 35S and subgenomic (20–25S) RNA species are found in cells infected with these viruses (Fan & Baltimore *J. molec. Biol.* **80**, 93; 1973; Gielkens *et al.* *Proc. natn. Acad. Sci. U.S.A.* **71**, 1093; 1974), but the 35S RNA species alone is found on polysomes immuno-

precipitated with antiserum to virus internal structural protein (Mueller-Lantzsch & Fan *Cell* **9**, 579; 1976). Direct translation of the two intracellular RNA species has recently been achieved by injection into *Xenopus* oocytes (Van Zaane *et al.* *Proc. natn. Acad. Sci. U.S.A.* **74**, 1855; 1977). The products, identified by immunoprecipitation, were shown to be internal structural proteins (*gag* gene) for the 35S RNA and envelope polypeptides (*env* gene) for the 22S RNA.

From these results it seems clear that the only 'open' cistron available for translation from the 35S genome RNA of oncornavirus is the *gag* gene, located nearest to the 5' end. Presumably the internal initiation sites for the other gene products are blocked in an analogous manner to the structural protein genes of togaviruses. Several problems

remain. The *env* mRNA can be identified as a discrete species in infected cells, but the mechanism of its synthesis by post-transcriptional processing or by some other means is not known. It may be relevant that subgenomic negative-strand RNA species which could be templates for mRNA synthesis have recently been identified in cells infected with RNA tumour viruses (Stavnezer *et al.* *J. Virol.* **20**, 342; 1976).

The mRNAs coding for the *pol* gene product or the *src* gene product have not been identified as yet. However, if the model proposed by Pawson *et al.* is correct, one possible way to get at the *src* gene product might be to use suppressor tRNAs to allow read-through of all the genes in 35S RNA from transformation-defective and non-defective viruses. □

## Amorphous semiconductors

from A. R. Long

The 7th International Conference on Amorphous and Liquid Semiconductors was held at Edinburgh on 27 June–1 July, 1977.

In the past, the study of amorphous semiconductors has been only loosely related to the wider field of study embracing the analogous crystalline materials. It has rather been dominated by ideas of electron localisation peculiar to disordered lattices. However, recent developments reported at the conference imply a significant change of emphasis, with a much stronger concentration on the non-crystalline analogues of well-known phenomena of the ordered state. This development was stimulated by the now well-documented discovery of the technique for doping amorphous silicon films prepared by the glow discharge decomposition of silane, to produce the familiar n-type and p-type materials and p-n junctions.

W. E. Spear and his coworkers (University of Dundee) presented a number of papers giving a coherent account of the electronic properties of doped amorphous silicon prepared by their glow discharge technique. Other groups, notably from IBM, Xerox, Harvard and the University of Chicago, working with similar material, emphasised the importance of hydrogen incorporation in these films in reducing the density of defect re-

lated electron states within the energy gap and thus enabling doping to take place. However, it is not possible to say at present if the substantial proportions of hydrogen contained in these films are themselves influencing the band structure of the material nor whether the doping process *per se* disorders the network further, thereby changing the intrinsic properties of the material as well as moving the fermi level. Much work remains to be done on these materials, particularly as they are of potential commercial importance.

The anomalous sign of the Hall effect observed for some amorphous semiconductors has long been a puzzle. D. Emin (Sandia Laboratories) described the manner in which the sign of the Hall coefficient is influenced by the connectivity of the appropriate structural units in the network and also the progress made in accounting for the magnitude and temperature dependence of the Hall mobility. With this work, an important technique in developing our understanding of crystalline semiconductors can now be applied with more confidence to the amorphous state.

The most striking recent theoretical advance in the physics of disordered materials has been the realisation, initially by Street and Mott, that the nature of electronic defect states may be profoundly influenced by the local atomic configuration, and in particular that pairs of singly occupied dangling bond states may in certain circumstances be energetically unstable and tend to form their charged analogues.

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These ideas may be used, as was reported in a number of papers, to explain a wide variety of low temperature photo-induced properties of chalcogenide glasses. Moreover, when interpreted within the chemical framework appropriate for chalcogenides, the 'valence alternation pair' model of Kastner, Adler and Fritzsche, they provide a convincing explanation of why it is not possible to move the fermi level in chalcogenides by doping in the traditional manner. Investigations were reported, by a group from Energy Conversion Devices led by S. R. Ovshinsky, of the modification of chalcogenide transport properties by the introduction of considerable atomic fractions of charged impurities into the matrix. However, the exact nature of the changes occurring in this modification process and the influence of the preparation conditions used are unclear at present, and it remains to be seen whether such modified chalcogenides will be of any lasting importance.

One interesting feature of the conference was the dominating position of certain lines of investigation and the virtual eclipse of topics which have dominated previous meetings. Little new structural work was reported, possibly because of the difficulty in interpreting unambiguously the results from traditional structural probes. However, the relatively new study of X-ray absorption fine structure (EXAFS), reviewed for the conference by D. E. Sayers (North Carolina State University) is an important advance, in that individual atomic species in multicomponent systems can be studied separately, and new results using this method were reported, particularly striking being the investigation of the coordination of arsenic impurities in amorphous silicon by the Xerox group. Recent studies of liquid semiconductors were restricted to two sessions, concentrating on conduction mechanisms. The thermal and vibrational properties of the disordered state which show highly anomalous features, were comparatively neglected, though important new results interpreting the dielectric properties of low temperature glasses in terms of polar two-level atomic states were presented by the Stuttgart/Grenoble group.

Papers concerned with applications of amorphous semiconductors were also few. David Adler (Massachusetts Institute of Technology), in a subtle and convincing analysis of a particular class of chalcogenide threshold switching devices, was able to demonstrate that thermal effects are insignificant and that electronic mechanisms are responsible for initiating and sustaining the on-state. Although there is undoubted interest in the fabrication of thin film photovoltaic cells for energy

conversion, little progress was reported beyond a definition of the design criteria, which will have to be met. It will be interesting to see if our enhanced understanding of basic processes can be turned into marketable devices by the time of the next international conference in this series.

From the papers presented at this conference, it is apparent that the study of amorphous semiconductors has progressed beyond the stage where uncertainties in the preparation conditions had a profound influence on observations. If an amorphous material is carefully prepared under well controlled conditions, and suitably characterised, it will in general be possible to make consistent measurements of its physical properties, which was by no means true in the early years of the subject. However, our theoretical understanding of many of the processes taking place in these materials is still weak, and the identification of the specific structural features responsible for particular effects almost non-existent. It is to be hoped that, with more reliable experimental data becoming available all the time, these deficiencies will soon be rectified. □

## Calcium and muscle regulation

from S. J. Smith

MUSCLE contraction involves the repetitive interaction of crossbridges from thick filaments with thin filaments, impelled by the hydrolysis of ATP. Although calcium is the universal intracellular signal which triggers contraction, there seem to be two targets for its action. In vertebrate skeletal and cardiac muscle, a subunit of the troponin complex on the thin filament is the calcium receptor; in the absence of calcium this complex impedes the interaction between actin and myosin. According to the currently favoured 'steric blocking' theory, calcium binding is accompanied by a movement of tropomyosin, a rod-like protein, which lies in the two grooves formed by the double helical array of actin monomers; the myosin-binding sites on actin are thereby exposed, allowing contraction to occur. In contrast, in invertebrates, regulation has been found to be linked to the myosin molecule, involving specific regulatory light chains. The most extensively studied system is that of the scallop, where the selective

release of one of the two chemically identical regulatory light chains by EDTA results in the abolition of calcium sensitivity of the actin-myosin interaction (Kendrick Jones *et al.* *J. molec. Biol.* **54**, 313; 1970). This is accompanied by the loss of half of the calcium-binding sites on the parent myosin. Calcium regulation is completely restored by replacement of the absent light chain in the presence of magnesium. This suggests that both light chains are necessary for regulation, and work by Szent-Györgyi *et al.* (*J. molec. Biol.* **74**, 179; 1973) implies that both myosin heads are cooperatively involved.

The advantages of the different types of regulation are not yet understood, and do not seem to be correlated with the speed of contraction of the muscle (see discussion by Lehman *Int. Rev. Cytol.* **44**, 55; 1976). In some phyla both regulatory mechanisms exist. The approach of Harris and Epstein (*Biochemistry* **16**, 859; 1977), who are investigating the contractile proteins of mutants of a 'dual-regulated' nematode worm, may prove valuable if mutants defective in one or other of the regulatory systems can be isolated.

There is no simple phylogenetic pattern for distribution of the regulatory systems. Myosin-linked regulation is not confined to invertebrates, but has been demonstrated in the smooth muscle of higher vertebrates (Bremel *Nature* **252**, 405; 1974). Recently, the mechanism of this regulation has been extensively investigated in chicken gizzard and pig stomach muscles (Sobieszek *Eur. J. Biochem.* **73**, 477; 1977; Small & Sobieszek *Eur. J. Biochem.* **76**, 521; 1977). It seems that the triggering of the actin-myosin interaction is brought about by calcium-dependent phosphorylation of the 20,000 dalton light chain by a specific light chain kinase. This occurs very rapidly and *in vitro* can be shown to precede the rise in actin-activated ATPase activity, suggesting that the process is physiologically important. Dephosphorylation occurs through the action of a phosphatase, which is not calcium sensitive and is present in approximately equivalent amounts to the kinase. The site of action of calcium is not yet certain; although smooth muscle myosin itself binds calcium in significant amounts, the isolated 20,000 dalton light chain does not, although it can be phosphorylated in the presence of calcium. This would suggest that calcium acts solely in the phosphorylation process but could also be consistent with a scheme whereby some interaction with the heavy chain is necessary for calcium binding by the light chain. The part that tropomyosin could play in a myosin-linked regulatory system was unclear. However, recently Sobieszek

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& Small (*J. molec. Biol.* **112**, 577; 1977) have shown that tropomyosin is necessary for amplification of the effect of phosphorylation, being required for the full development of actin-activated ATPase activity. It would seem that, on calcium activation, as in skeletal muscle (reviewed by Weber & Murray *Physiol. Rev.* **53**, 612; 1973), tropomyosin brings about a cooperative change in the actin filament, which facilitates the actin-myosin interaction.

Whereas myosin-linked regulation in smooth muscle now seems to be indisputable, it is not certain whether troponin is present in these muscles. Ebashi reports (Mikawa *et al.* *J. Biochem.* **81**, 273; 1977) an 80,000 molecular weight calcium-sensitising factor, which has no kinase activity. Moreover troponin C, the calcium-binding component of the complex, has been isolated from uterus muscle by affinity chromatography using troponin I, the inhibitory unit of the skeletal muscle complex (Head *et al.* *Biochem. J.* **161**, 465; 1977). However, *in vitro* functional tests suggest that there is no thin filament regulation (Sobieszek & Small *J. molec. Biol.* **102**, 75; 1976). It seems possible that this calcium-sensitising factor performs some other, as yet unrecognised function in smooth muscle. In this context, it is of interest that a troponin C-like protein, which will restore calcium sensitivity to desensitised skeletal actomyosin, has been isolated from brain, fibroblasts and neurosecretory tissue, where it seems to modulate cyclic nucleotide turnover. (Amphlett *et al.* *FEBS Lett.* **72**, 163; 1976).

In the light of these new findings of calcium-dependent phosphorylation as the trigger for actomyosin ATPase activity in smooth muscle, it seems valuable to consider the possibility of myosin-linked regulation in skeletal muscle. A calcium-sensitive light chain kinase, distinct from that in smooth muscle, has been demonstrated in skeletal muscle, but no link between phosphorylation and actin-activated ATPase activity could be found (Perrie *et al.* *Biochem. J.* **135**, 151; 1973). There are suggestions that phosphorylation and the calcium response of skeletal myosin may be intimately related (Okamoto & Yagi *J. Biochem.* **80**, 11; 1976). Until recently, there was no *in vitro* evidence for calcium regulation by skeletal myosin. However, Lehman (*Biochem. J.* **163**, 291; 1977) has demonstrated that this can occur in some conditions.

All the evidence so far accumulated indicates the complexities of control mechanisms potentially available for the precise regulation of excitation-contraction coupling; an assessment of their physiological importance will provide a step further towards the seem-

ingly ever-receding mirage of a complete understanding of muscular contraction. □

## Cosmic ray age, soft X-rays and the galactic wind

from J. J. Quenby

ALTHOUGH the distribution of stars in our Galaxy is in the form of a thin disk, a strong flow of plasma perpendicular to the plane may produce a magnetic halo of near circular shape. Recent cosmic ray balloon measurements carried out by Webber, Lezniak, Kish and Simpson of the University of New Hampshire (*Astrophys. Lett.* **18**, 125; 1977) may provide data which aids the modelling of the phenomena involved.

Initial experimental observation of the presence of a galactic halo which follows the figure of an ellipsoid of revolution come from cosmic radio noise data at hundreds of MHz. Relativistic cosmic ray electrons, spiralling in weak, few microgauss or less magnetic fields, are thought to produce the radio noise by way of the synchrotron radiation process. The halo constitutes a possible magnetic reservoir for cosmic rays and these particles could therefore spend the greater part of their lives well outside the galactic disk.

A recent, dynamical argument for the existence of the halo has been put forward by Ipavich (*Astrophys. J.* **196**, 107; 1975). He starts from the idea that the anisotropic motion of cosmic rays along the interstellar magnetic field lines may generate and amplify magnetohydrodynamical waves by a scattering process, but the waves in turn lose energy to the interstellar gas. The net result is that cosmic rays heat the interstellar medium and a situation, analogous to the solar wind in the solar corona, may arise. An out-of-the-galactic plane flow is initiated which eventually becomes supersonic in a manner similar to the sub to supersonic transition at the neck of a rocket motor nozzle. Gravity perpendicular to the disk plays the same part as the constriction in the motor nozzle. Hence interstellar field is carried out by this galactic wind to constitute an additional, low density medium reservoir for the cosmic rays.

Studies of the relative abundances of the cosmic ray isotopes (Li, Be, B) known to be produced by nuclear inter-

actions in the interstellar medium suggest that the mean amount of matter transversed by the cosmic rays is  $\sim 5 \text{ g cm}^{-2}$ . Taking this figure in conjunction with the  $\sim 1 \text{ cm}^{-3}$  number density of interstellar atoms implies a cosmic ray life of about  $10^6$  yr. An alternative and more direct measure of this life lies in finding the amount of radioactive  $^{10}\text{Be}$  relative to its decay product  $^{10}\text{B}$  existing in the cosmic ray flux. The  $1.5 \times 10^6$  yr beta decay half life of this process is conveniently matched to the expected cosmic ray age. Spacecraft telescopes flown by Garcia Munoz *et al.* (*Astrophys. J. Lett.* **201**, L141; 1975) detected few or no  $^{10}\text{B}$  particles, suggesting a most probable age  $\sim 2 \times 10^7$  yr. These workers reconcile their age measurement with the order of magnitude smaller apparent value given by the Li, Be, B abundances by supposing that cosmic rays spend most of their life in the halo where the matter density may be one hundredth of that in the disk. Unfortunately, conflicting experimental evidence is available from a much larger, but balloon borne, detector flown by Hagen *et al.* (*Astrophys. J.* **212**, 262; 1977) which recorded enough  $^{10}\text{Be}$  to be consistent with an age of only  $5 \times 10^6$  yr. Webber and coworkers have now performed a balloon experiment in a similar energy range to that of Hagen *et al.* but at a greater altitude so that fewer unwanted atmospheric interactions take place. Also this latest detector seems to achieve better mass resolution than that of previous balloon experiments. Webber *et al.* carefully consider the distortion effects of solar modulation on the relative  $^{10}\text{Be}$  abundance (see *News and Views* **259**, 11; 1976) and find that the mean cosmic ray life must be at least  $8 \times 10^6$  yr and could be  $15 \times 10^6$  yr or more. They conclude that their data would allow either a disk or halo model for cosmic ray confinement.

Owens and Jokipii (*Astrophys. J.* in the press) have set the halo model for cosmic ray motion within the context of Ipavich's work. They show that a cosmic ray age of more than  $10^7$  yr requires a halo scale height of 4 to 10 kpc and an average galactic out-of-plane wind velocity of  $< 60 \text{ km s}^{-1}$ , numbers consistent with Ipavich's estimates. It is interesting to note that Ipavich also requires an interstellar gas temperature in the  $10^4$  to  $10^6$  K range. Soft X-ray background survey data points to a hot galactic gas temperature  $\sim 6 \times 10^5$  K as revealed by rocket measurements made by Levine and coworkers at MIT. Further insight into the dynamics of a hot galactic gas can arise from a consideration of the X-ray emission from rich clusters of galaxies such as COMA. The soft X-ray line and continuum data on the

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COMA cluster are consistent with a model in which a hot, intercluster gas receives its energy from heating inside the individual active members of the cluster (see Serlemitsos *et al. Astrophys. J. Lett.* **211**, L63; 1977 for experimental data; Yahil *et al. Astrophys. J.* **185**, 787; 1973 for theoretical discussion). Cosmic ray heating could well be the relevant non-thermal process feeding the intercluster medium through enhanced galactic winds. □

## Forecasting in crop protection

from R. Hull and C. R. B. Baker

The Second Conference on Forecasting in Crop Protection was held in Paris on June 21–24 by the European and Mediterranean Plant Protection Organisation.

THE ability to forecast reliably the timing and intensity of pest and disease attack on crops is crucial to reducing unnecessary and undesirable use of pesticides if at the same time agricultural productivity is to be maintained and improved. This claim was made for many national forecasting schemes described at the conference, which covered national forecasting organisations, meteorology, field monitoring methods for pests and diseases, interpretation of pest and disease counts, development of forecasts, and the link-up with the growers.

Schemes of varying sophistication were outlined. They ranged from a computer-based system organised by Michigan State University in the US, through national networks of observers and warning stations operated in countries such as France and Hungary, to systems designed to provide warnings of a specific pest or disease. Techniques on which forecasts are based include monitoring pests and diseases by sampling crops, trapping insects in pheromone traps and in tall suction traps that sample the migrant fauna in the air, observations on caged pests, observations on plots of appropriate crops maintained at regional forecasting centres and the use of meteorological data. In most countries such schemes are operated by government agencies or are subsidised by government funds. Since the activity of all organisms damaging crops is influenced

in one way or another by weather, much attention was given to recording and interpreting meteorological data in relation to phytopathological problems.

Means of communication with farmers and growers included postal warnings, radio and television broadcasts, telephone answering services, public notices and personal contact. Although delegates seemed convinced that their own national schemes were making some impact on farming practice, especially in reducing use of pesticides, little quantitative evidence was presented that pests and diseases were being controlled more efficiently or that the forecasting schemes were economic. It was emphasised that forecasts were generally issued to cover a region and cannot be detailed enough to show the local variations that affect the individual grower. He needs local advice or further education so that he can make his own judgement based on the growth stage of his own crops and the actual stage and abundance of the pests or diseases present and also sometimes of their natural enemies.

Several speakers stressed the need for a thorough knowledge of the biology of pests and diseases, but important gaps in such knowledge were

evident throughout the conference. In particular there was a lack of a coherent picture of differences in pest behaviour across Europe. Standardisation of assessment methods as recommended in the FAO manual on Crop Loss Assessment Methods was discussed.

Agricultural research and advisory services have been operating in most developed countries for several decades. It was clear from this conference that despite all the effort applied so far, problems in pest and disease management continue and that forecasting is an essential part of any ultimate solution. Unless flexible, the best devised research and advisory programmes could be nullified by a change in agricultural practice, such as that from ploughing to direct drilling.

The conference made two proposals for follow-up action under the aegis of EPPO. First, a working party should be convened to appraise the various systems of assessing and forecasting crop losses. Second, closer cooperation should be organised between meteorological and plant protection services in the collection, dissemination and use of meteorological observations in relation to the needs of crop protection. □

## Tree-ring dating in Britain comes of age

from a Correspondent

The first International Symposium on Dendrochronology in Northern Europe was held at the National Maritime Museum, Greenwich, London from 11 to 14 July, 1977. The papers will be published by *British Archaeological Reports* (112, Banbury Road, Oxford OX2 7BP) in collaboration with the National Maritime Museum and the Research Laboratory for Archaeology and the History of Art, University of Oxford. A companion exhibition, instigated by Dr J. M. Fletcher (University of Oxford), on tree-ring analysis of Tudor portraits is open at the National Portrait Gallery, London until 18 September 1977.

In the historic setting of the National Maritime Museum close by the Royal Observatory, Greenwich Park—long famous for the measurement of British Standard Time—the precise measurement of the annual growth rings of trees for dating purposes was shown at the meeting to be an interdisciplinary and international subject with great potential for European environmental science and archaeology. Dendrochronology, although not yet a commonplace technique, already is

shedding light on such wide ranging subjects as past climates, sea level changes, tree ecology and forest management in prehistoric and historic times, and is proving an invaluable aid in dating of archaeological remains and in art history.

Holding the meeting in London was particularly important for until the past 10 years or so little research on tree-ring analysis has been done in Britain, although some pioneering work was done in the 1940s and 50s by Lowther and Schove. This slow start may be explained partly because of the difficulty of devising suitable reference chronologies for oaks growing in southern and eastern England, but also partly by unfamiliarity with the classical German and Russian work on the subject.

The problem in the development of tree-ring analysis in north-west Europe has been that the growth of deciduous trees and thus the pattern of annual rings is more variable in temperate regions than in the semi-arid and arid areas of south-west USA: site conditions (such as altitude, and soil), defoliation by insects and man's influence may affect tree growth in Europe as much as temperature and rainfall—the predominant

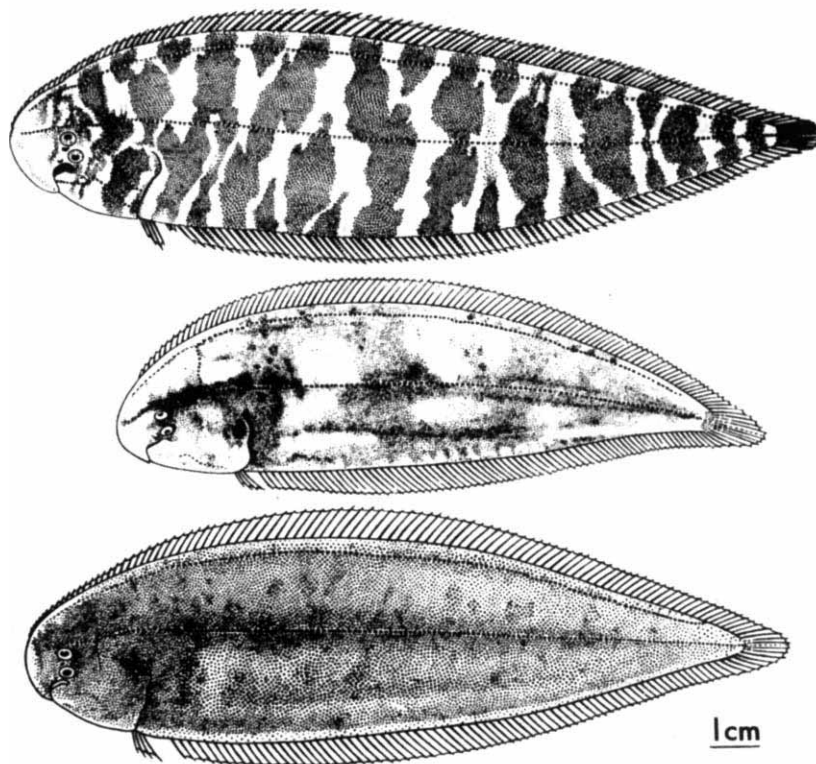
R. Hull has just retired as Director of Broom's Barn Experimental Station, Harpenden; C. R. B. Baker is an entomologist at the Plant Pathology Laboratory of the Ministry of Agriculture, Fisheries and Food at Harpenden.

## Tongue soles revised

from our Marine Vertebrate Correspondent

THE flatfishes are an interesting and important group of bony fishes: interesting because of their unique asymmetry which involves a complicated reordering of the head skeleton, eyes and optic nerves on metamorphosis; important because almost throughout the world they are of considerable value as food fishes. These two aspects have resulted in their being studied by workers in different disciplines possibly more than most comparably sized fish groups. Their systematic arrangement and taxonomy have been studied, notably by Norman in his *Systematic Monograph of Flatfishes 1* (London, 1934) although he never completed the second volume which was to have included the families of true soles and tongue soles (Soleidae and Cynoglossidae).

To some extent this hiatus has partly been filled by a comprehensive revision of the tongue soles of the genus *Cynoglossus* by A. J. K. Menon (*Smithsonian Contributions to Zoology*, no. 238, 1-129; 1977). The Cynoglossidae are flatfishes which have a typical elongate body form, tongue sole being a most apt name. The dorsal, caudal, and anal fins are continuous around the edge of the body, other fins are reduced or absent, and the mouth is placed on the side of the head, in most species having a sinister curve about it. In the genus *Cynoglossus* the eyes are very small, close together and are always on the left side of the head. It seems that the reduction of the eyes is probably associated with the well-developed lateral line sensory system; in some species three lateral lines run along the length of the body while most have a complex of head lines. On the blind side, however; this sensory system is reduced or totally absent (a striking difference to the situation in the true soles which have a highly evolved sensory system on the eyeless side of the head). As Menon points out members of the Soleidae are mostly feeders on the surface of the sea bed, while the tongue soles are adapted to plough through the mud or sand on the bottom, hidden beneath the surface themselves, and feeding on buried food organisms.



Three specimens of *C. puncticeps* showing variation in pigment pattern. Top from Kerala; others from Cochin.

As an adaptation to this way of life the cynoglossids do not have the fin rays of the dorsal fin running forward to the extreme edge of the head (as do the soles) but the first dorsal pterygiophore, which in other fish would support the anterior fin rays, is modified to form a rostral cartilage. This rostral cartilage provides support for the thin, elongate snout which acts like a forward-pointing ploughshare as the fish slices its way through the seabed.

Menon's revisionary study is, of course, basically a systematic work. He gives a brief discussion of the other genera in the family (*Symphurus* with forty or so species, *Paraplagusia* with only four species), and then detailed diagnoses of the forty-nine members of the genus *Cynoglossus*. All the species are illustrated by simple line drawings supplemented by photographs of most species. Menon's work thus means that in the future there will be an adequate source of identification available for these closely similar fishes.

The geographical distribution of

these tongue soles has a number of points of interest. The centre of distribution lies in the eastern Indian Ocean-West Pacific area, especially around the Malay archipelago, where Menon finds the more specialised and rapidly evolving species complexes. Around the periphery of this region more primitive types are found, ranging from Japan to eastern Australia, and along the Indian Ocean coast of Africa. *Cynoglossus* is one of the few groups of fishes which are widespread in the Indo-Pacific and are found off the West African coast as well, although absent in the Western Atlantic. Most of the West African species belong to a large group of species (the *canariensis* group) which Menon regards as primitive and near to the ancestral stock. Menon's study thus has some interest in the evolution of this group of flatfishes and will be of value to zoogeographic studies although work in a broader field than at generic level will be necessary to evaluate some of his conclusions concerning relationships and distribution.

factors in arid and tropical countries—and so make it difficult in parts of northern Europe to devise reference curves for trees such as oak, beech and elm which all react differently to environmental conditions. In northern Europe too there is the added disadvantage that no tree has the lon-

gevity of the bristle-cone pine or sequoia although sessile oaks often live more than 500 years. Oak is the timber found most often in archaeological excavations.

Although the Russian, Shvedov, as long ago as 1892, related variations in annual ring width to rainfall, it

was the German, Huber, who from the 1940s to his death in 1970, laid the foundations of the modern methodology and his influence remains in the rigorous work of J. Bauch and D. Ekstein (University of Hamburg) and B. Becker (University of Hohenheim) which sets standards for all other



laboratories. Computer programs devised by Bauch and Ekstein now make it possible to cross-date local and regional tree-ring curves reliably. A master chart established for oak in the Netherlands dating back to AD 1036 synchronises with corresponding curves in southern and western Germany where, as Becker reported, there is now a master chronology from the present back to 717 BC.

In northern Germany and in the coastal region of the North Sea from Denmark past the Netherlands and Flanders to south-east England and East Anglia, however, the major German chronology does not usually apply and so independent curves have been constructed. For Hainabu in the Schleswig area of north Germany, where dendrochronology has been used to date the important Viking trading centre, Ekstein has established a chronology dating back to AD 400. In the Netherlands Bauch has found a second chronology, independent of his other, which covers the years AD 1109–1637. To this curve are related parts of eight overlapping chronologies, each about 300 years long and covering the period AD 514 to 1609, established by J. M. Fletcher (University of Oxford) for southern and eastern England.

Both Fletcher and Bauch have used a combination of excavated and architectural timbers and oak panels of paintings to make their reference curves. An added dimension to this work is its relevance to art history. In England, for example, oak panels were the common means of support for most portraits painted before 1620. By classifying 150 such paintings dendrochronologically, Fletcher has been able to gather information about panels from the same tree and to deduce the likely date to within 10–20 years of some of the portraits whose history is uncertain. Bauch, similarly, has studied over 200 Dutch and German paintings mainly of the seventeenth century.

Other chronologies for Britain are gradually being established. P. Leggett and A. Hibbert (Liverpool Polytechnic) are working on trees felled in woodland in North Wales and have a master chronology for oak running from AD 1711 to 1975 which cross-dates with one in Northern Ireland prepared by M. G. L. Baillie (Queen's University, Belfast). R. Morgan (University of Sheffield) is dating material excavated by J. Schofield (London Museum) from Roman and Mediaeval sites on the River Thames in London. Tree-ring analysis of this timber, recovered from old waterfronts, is helping to clarify discrepancies between the dates inferred from the stratigraphical position of artefacts found on the site

and radiocarbon dating. Valuable topographical clues are also being obtained from the way the timber was used in the construction of the waterfronts, and changes in the environmental conditions around London from Roman to Mediaeval times can be suggested by study of the ring-widths of the oak timbers. When two species, oak and elm, are found together in Mediaeval excavations, then comparisons of their use and growth at a particular time have been made (D. W. Brett, Bedford College, London). In another part of England an oak chronology for the period AD 1635–1972 has been established for the Winchester area (A. C. Barefoot, North Carolina State University, and others) and this curve, when combined with  $^{14}\text{C}$  dates and a separate chronology for earlier timber, is being used to date excavations in the city led by M. Biddle (Winchester Research Unit).

Across Europe in East Germany, M. Jährig (Akademie der Wissenschaften der DDR, Berlin) has examined pieces of timber excavated from Slavonic settlements dating from the 6th to the 10th centuries AD with the aim of establishing a master chronology for GDR. So far he has obtained a relative chronology for 492 years in this period in the central region of the country; a separate chronology will probably be needed for the northern region.

In Sweden, there is now a master chronology for oak for the past 1,400 years. Analysis of oak used to make coffins excavated from Mediaeval churchyards in Lund (T. S. Bartholin, University of Lund) is suggesting how the climax forest in that part of Scandinavia changed during the period AD 1000–1200. In the earlier coffins the oak boards are narrow ringed and range up to 500 years old; but by AD 1150 the oaks had few and broad rings, suggesting that by this date open woodland pasture had replaced the dense virgin forest.

Much earlier chronologies using timber and radiocarbon dates are being devised for the Somerset Levels, a wetlands area in south-western England where an extraordinary series of wooden trackways of various constructions have been excavated by an inter-university team led by J. M. Coles (University of Cambridge). A member of this team, B. J. Orme (University of Exeter), described how tree-ring analysis is being used to establish dates for the substantial oak planks and for the brushwood material of ash, hazel, alder and birch used in the building of the trackways which date from the fourth to the first millennium BC. Analysis of this worked wood at different sites has enabled the team to determine which woods

were used for which purpose in the trackway structures, to show that woodland management by coppicing existed as long ago as the fourth millennium BC and to ascertain the durability and re-use of different timbers. Past environmental conditions can also be inferred from studies of the timber from different millennia.

Other applications of dendrochronology reported included a study of submerged forests around the British Isles by A. Heyworth (University College of North Wales, Aberystwyth) who is using tree stumps ranging in age from more than 8,000 years to less than 1,000 years as a measure of sea level changes. R. E. Tout and W. E. Gilroy (University of Surrey) are analysing concentrations of trace elements in tree rings: the technique offers a way of distinguishing the heartwood and sapwood boundary where this is unclear. The elements potassium, chlorine, and manganese seem useful as indicators.

Most dendrochronologists use measurements of ring width to prepare their reference curves. Another line of approach is to measure wood density by X-ray densitometry and two speakers, F. H. Schweingruber (Swiss Forest Research Institute, Birmensdorf) and H. Polge (Centre National de Recherches Forestières, Champnoux, France) believe that the technique is a better indicator than ring width of climatic changes in the past and is quicker to use. Schweingruber is using subalpine conifers (fir, spruce, pine and larch) to reconstruct the fluctuations in summer temperature in the Alps. S. J. Milsom and M. K. Hughes (Liverpool Polytechnic) are experimenting with X-ray densitometry on oak which seems to present more problems than pine although it is thought that the technique will be useful for dating wide ringed timber.

On the third day of the meeting, the point made by G. C. Varley (University of Oxford) that defoliation of oaks by caterpillars can have similar effects on tree growth as harsh weather led to lively discussion and reminded everybody that the wood they work with came from a living tree whose growth was influenced by a variety of quantifiable and unquantifiable factors, not forgetting drought, plagues of insects, sunspots and other 'Acts of God'. These may be 'noise' but should not be disregarded in constructing computer programs. Computers and statistics, although essential tools today, must be used discriminately; as several participants pointed out the human eye and brain remain indispensable for noting anatomical irregularities in timber and for the matching of tree-ring curves. □

# review article

## Radio sources with superluminal velocities

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*Radio data from four extragalactic sources, three quasars and one galaxy, show evidence for an apparent expansion faster than the speed of light. The data on these 'superluminal' sources are reviewed, and their implications briefly discussed.*

BRIGHTNESS distributions in four extragalactic radio sources have been seen to vary so rapidly that the apparent transverse velocity of expansion is greater than the velocity of light (assuming a cosmological origin for the redshift). The term superluminal will be used to describe this phenomenon. In this paper we review many of the observations of superluminal expansions, and also add some new material. Blandford, McKee, and Rees<sup>1</sup> have summarised theoretical ideas on the subject.

The observations have all been obtained with very-long-baseline interferometry (VLBI) systems using two to five radio telescopes, spaced over thousands of kilometres, to form multi-element interferometers. Various model-fitting and map-making procedures have been used to estimate parameters of the brightness distribution, such as diameter, separation and flux of components, position angle, and so on. In some cases the data have been sufficiently accurate and extensive to warrant many-parameter models, and detailed contour diagrams have been made. In general, however, the models are not unique. Some have been challenged<sup>2,3</sup>; but there still is general agreement among different workers on the main features of the sources. VLBI map-making is presently in a stage of rapid development; in particular there is a growing use of phase data, and more definitive results are expected in a few years.

Four sources including three quasars (3C345, 3C273, and 3C279) and one radio galaxy (3C120) will be discussed. In each case we give simple angular measures and do not discuss the detailed models of brightness distribution. More complete discussions will be found in the references.

### 3C345 ( $z = 0.595$ )

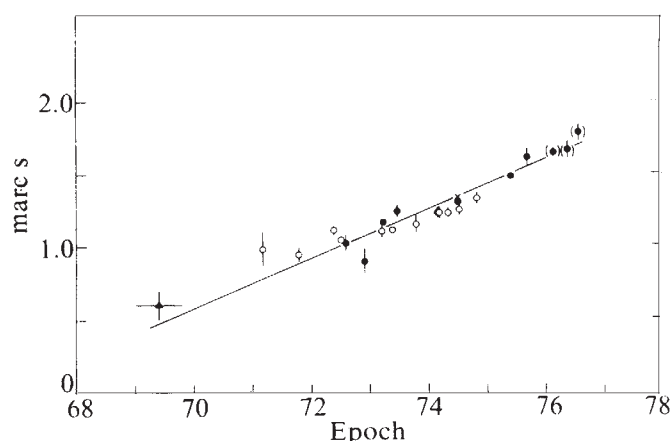
Two observing groups<sup>4,5</sup> have independently shown that 3C345 could be accurately described as a simple double source in mid-1974. The two components were approximately Gaussian and contained most or all of the flux density at  $\lambda = 2, 2.8$ , and 3.8 cm. In these experiments three or four telescopes were used. The simple model was an excellent fit, and there was no requirement for, nor evidence of, a more complex structure. At most

other epochs only two or three telescopes were used. The brightness distributions were not as well determined, of course, but a simple double fit the data and the separation,  $\theta$ , was accurately determined in every case.

Figure 1 shows the separation of the centres of the two components as a function of time. The point at 6 cm is taken from a number of measurements made in 1968 and 1969 (ref. 8). The source was only slightly resolved at that early epoch and  $\theta$  was determined from the best-fitting double. The position angle was assumed to be  $105^\circ$ , the value found more recently. The 3.8 cm point at 1971.1 (ref. 7) is similarly model dependent because the source is only partially resolved. The three points in 1976 are provisional because detailed model fitting is yet to be done; however, they are unlikely to change much because  $\theta$  is well determined from the sharp minima of the visibility function.

The points in Fig. 1 show that the brightness distribution expanded by a factor of about 3 in 7 years, at the approximate

**Fig. 1** Apparent separation of the two components of 3C345 as a function of time. The slope of the line is  $0.17 \text{ marc s yr}^{-1}$ . The observations at 2 cm are taken from ref. 5; the 2.8-cm points are from refs 5 and 6, and the four points in 1972–73 are from private communications with N. Broten and M. Quigley (the points in 1976 are discussed in the text); the 3.8 cm points are from refs 4 and 7; and the 6-cm point is discussed in the text.  $\times$ , 2.0 cm;  $\bullet$ , 2.8 cm;  $\circ$ , 3.8 cm;  $\blacktriangle$ , 6.0 cm.



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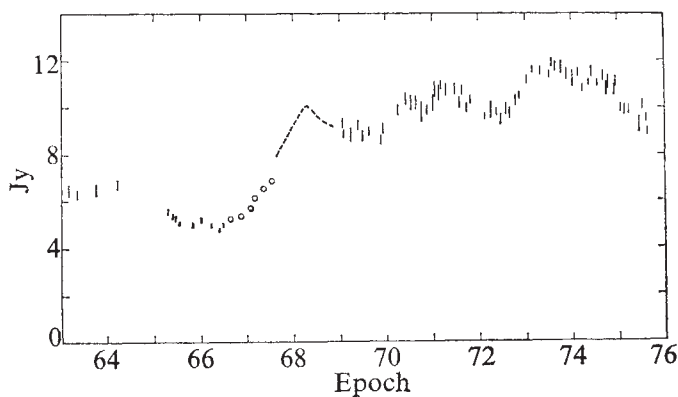


Fig. 2 Total flux density of 3C345 at  $\lambda = 3.8$  cm. Points from 1963 to 1966.5 are taken from ref. 10; from 1966.6 to 1967.5, ref. 11; 1969.0 to 1971.6, ref. 12; 1971.6 to 1974.4, ref. 13; 1974.4 to 1975.6 from private communications with W. A. Dent, and the dashed line is interpolated between observations at 2.8 and 4.5 cm reported in ref. 14.

rate 0.17 marc s per year. The expansion may not be uniform, however, for the slope seems to be flatter in 1972–73, and steeper in 1975–76. The difference in slope explains most of the discrepancy in the rates already reported in the literature<sup>6,9</sup>. The angular rate can be converted into an apparent transverse velocity at the source by multiplying by the cosmological distance and correcting for time dilation with a factor  $(1+z)$ .

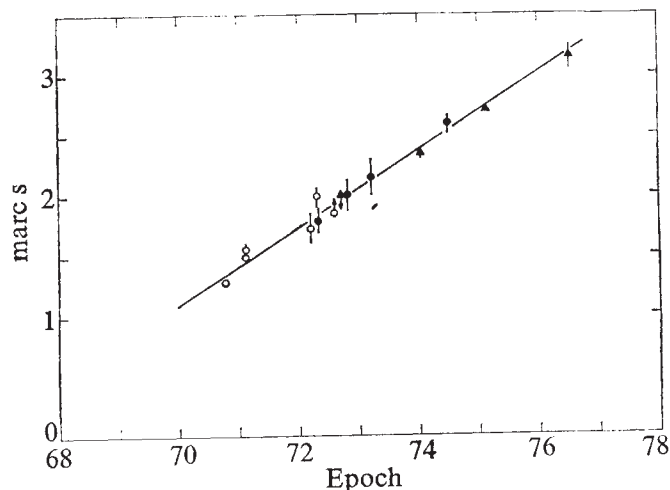
$$\frac{v}{c} = \frac{\theta}{H_0 q_0 (1+z)} (q_0 z + (q_0 - 1)[(1 + 2q_0 z)^t - 1])$$

The line in Fig. 1 corresponds to  $v/c \approx 7$ . ( $H_0 = 55 \text{ km s}^{-1} \text{ Mpc}^{-1}$  and  $q_0 = 0.05$ .)

The angular separation of the components of the double seems to be independent of wavelength over at least an octave. However, the detailed visibility curves show that the ratio of flux densities varies with wavelength; that is, the components have different spectra.

The position angle (PA) of the double was determined for all cases shown in Fig. 1 for which there were adequate data. There

Fig. 3 'Size' of 3C273, where size  $\theta^* = (2w)^{-1}$  and  $w$  is the distance to the first minimum of the visibility function. The slope of the line is  $0.32 \text{ marc s yr}^{-1}$ . The observations at 2.8 cm are taken from refs. 15 and 16 and the points at 3.8 cm are taken from refs 7 and 17–19. The 6-cm points are our previously unpublished data. ●, 2.8 cm; ○, 3.8 cm; ▲, 6.0 cm.



are no substantial differences with wavelength or time; the PA has been constant at  $105^\circ \pm 3^\circ$ .

Extrapolation of the rate shown in Fig. 1 to zero separation indicates that an 'event' occurred in 1966. Figure 2 shows the total flux density of 3C345 at 3.8 cm. A major increase in flux density began in 1966; the flux doubled in two years and has since stayed high. The near coincidence of the times suggests that the outburst and the expansion have a common origin.

### 3C273 ( $z = 0.158$ )

3C273 has a complex elongated structure (refs 3, 15 and 16 and W. D. Cotton *et al.*, in preparation). Its low declination ( $2^\circ$ ) means that the  $(u, v)$  coverage is poor and the models are more indeterminate than for high declination sources like 3C345. In consequence, different authors have proposed models of different character, some expanding with time<sup>7,15</sup>, and others with stationary components whose intensities are suitably varied<sup>3,17</sup>. All observers agree that the overall size of the brightness distribution increased from 1971 to 1974, but the uniformity and nature of the increase has been argued.

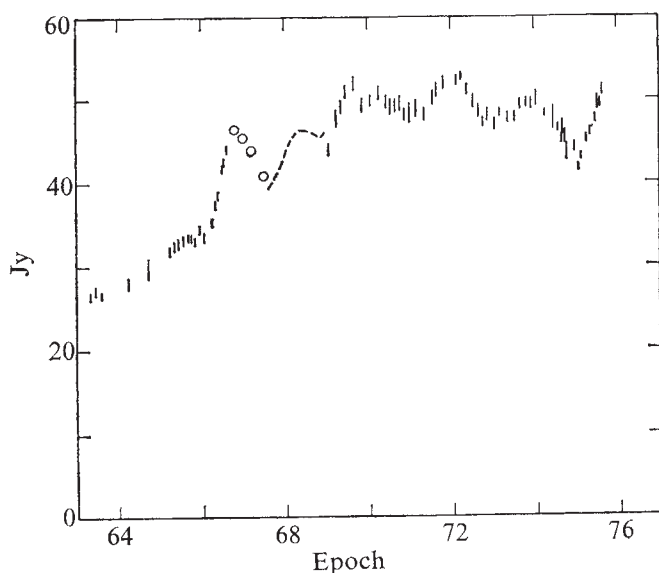


Fig. 4 Total flux density of 3C273 at  $\lambda = 3.8$  cm. References for the points are as in Fig. 2.

Figure 6 of Schilizzi *et al.* (ref. 15) shows a succession of  $(u, v)$  diagrams with the observed lines of maxima and minima of the visibility function. The lines are not equally spaced; therefore, the source cannot be represented by a simple (that is, Gaussian) double. Furthermore, the relative spacings change with time, and successive sets of lines move toward the origin. This means that the shape changes with time, and that, in some measure, it is increasing in size. The visibility data shown by Schilizzi *et al.*, however, do not define the brightness distribution uniquely.

In an attempt to escape difficulties caused by modelling with inadequate data, we have studied one simple measure of the overall size, the distance from the origin to the first minimum of the visibility function. Let  $w$  (in wavelengths) be this distance, then  $\theta^* \equiv 1/(2w)$  is the angular separation of the components if the source is a simple double. In the more general case where the source consists of several isolated components strung out on a line,  $\theta^*$  is a good measure of the overall angular scale. For example, in a wide range of triples which we studied numerically,  $\theta^*$  varies from 1.0 to 0.65 times the overall size.

We plot  $\theta^*$  at various epochs in Fig. 3. Many of these observations were taken on only one baseline, and the position angles were very poorly determined. So, to provide a consistent basis for calculating  $\theta^*$ , we assumed  $\text{PA} = 64^\circ$  in all cases. This is the typical value obtained when multi-baseline data are available, although the source is known to be nonlinear<sup>3,16</sup>. The error bars

in Fig. 3 come from the uncertainty in estimating the locations of the minimum, and do not include any errors from an incorrectly assumed position angle. The arrows in mid-1972 signify lower and upper limits.

Further data exist at 2.8 cm for 1975 and 1976, but the character of the visibility function is different then from what it was earlier. The functions do not contain any clearly recognisable extrema (except at the origin), but drop rather smoothly to a 'core' value. The brightness distributions may still contain 'components', but all (or perhaps all but one) of them must be large enough to be individually resolved at the interferometer spacings which would otherwise produce maxima and minima. In contrast, at 6 cm the structure still had concentrations which could produce the observed minima. These recent results will be discussed separately.

The 2.8-, 3.8-, and 6.0-cm data all fit together very well up to 1975. We conclude that the basic shape was independent of wavelength over this range, although there were spectral differences in component intensities.

The overall size of the brightness distribution increased rather steadily and more than doubled in 6 years. The word 'expansion' may properly be used to describe this behaviour. The points in Fig. 3 cluster around a line with slope  $\theta^* = 0.32$  marc s per year ( $v/c = 4.2$ ). If the line is extrapolated it hits zero in 1966. The total flux density at  $\lambda = 3.8$  cm is shown in Fig. 4, and, again, the 'expansion' starts, roughly, near the beginning of a large sustained increase in flux density.

### 3C120 ( $z = 0.033$ )

3C120 varies rapidly in flux density and appearance, and has been observed frequently in an attempt to follow its variations<sup>7,15,21-25</sup>. Although many of the observations have been rather incomplete, in several cases very good data were obtained with 3 or 4 telescopes, and simple double models fit very well. The best of these cases comes from our observations at 2.8 cm in 1976.1, when 5 well defined maxima and minima were observed using telescopes at Green Bank, Fort Davis and Owens Valley. As with 3C345, a well-separated Gaussian double accurately fit all the visibility data. However, the compact structure in 3C120 never produced nearly all the total flux, the way it did in 3C345. The compact components of 3C120 are embedded in a larger, undetermined structure.

In all cases with good data the PA was near  $65^\circ$ . To determine a size in the poorer cases, we assumed that a double was always a reasonable representation of the source; and, when necessary, we also assumed PA  $= 65^\circ$ .

The separation of the two components of the double is plotted in Fig. 5 for all values in the literature (with exceptions noted below) together with some of our previously unpublished data at 2.8 and 6.0 cm. The parentheses indicate cases where there was ambiguity; typically, the source was only weakly resolved, or the size determination was made from a single minimum on one baseline, or the first minimum was not directly observed.

We recognise two major epochs of expansion in Fig. 5, and have indicated them with straight lines whose slopes correspond to  $v/c = 5$  and  $v/c = 8$ . At 1974.5 the 3.8 and 2.8 cm observations showed doubles of very different separation. No unique interpretation can be given for this, for the size and strength of the components of the doubles are very poorly determined. The observed double structure did not account for the total flux at either wavelength, so it is possible that both doubles existed at both wavelengths but were not recognised because of the differing resolutions. Thus, the close-spaced double seen at 2.8 cm might not have been recognised at 3.8 cm because its first minimum would have come at  $1.9 \times 10^8$  wavelengths, whereas the maximum length of the baseline was only  $1.0 \times 10^8$  wavelengths. Similarly, at 2.8 cm where the minimum length of the baseline was  $1.6 \times 10^8$  wavelengths, components larger than about 1.5 marc s would have been resolved, and this double would have been missed. Spectral differences of the type apparently seen in 3C273 would increase the size of the outer components at 2.8 cm and accentuate this behaviour.

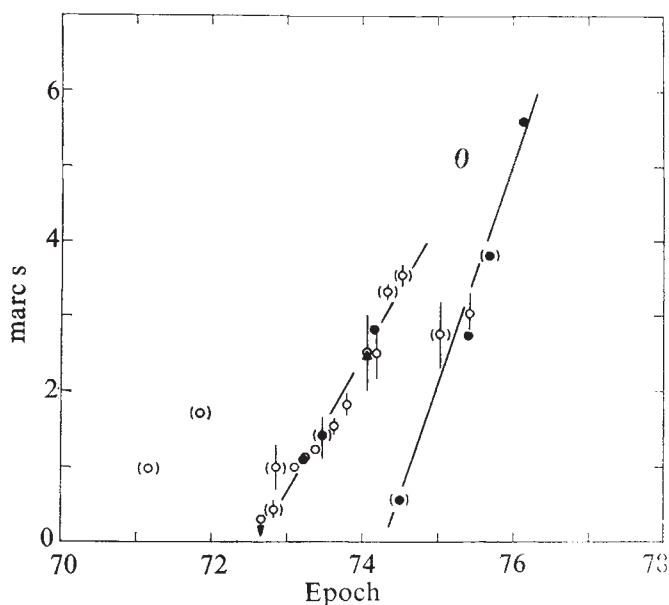
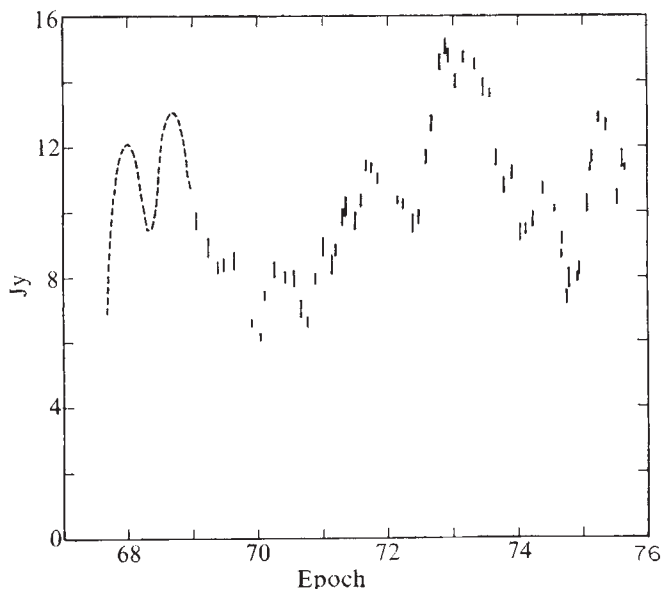


Fig. 5 Separation of the two components of 3C120, assuming a double at PA  $= 65^\circ$  when necessary. The slopes of the lines are 1.8 and 2.9 marc s yr<sup>-1</sup>. The early observations at 2.8 cm are from ref. 15, and the points from 1974.5 on are previously unpublished. The 3.8-cm data are from refs 7, 15, 21-25. The 6-cm point at 1974.5 is previously unpublished. ●, 2.8 cm; ○, 3.8 cm; ▲, 6.0 cm.

The points in 1971 were once interpreted as evidence for a superluminal expansion<sup>21</sup>, but that result is ambiguous with only two points. Further data exist for early 1972 (refs 22, 23), but they are not plotted. The visibility was changing rapidly then, but remained very low. Therefore, the strong concentrations which must have existed in 1971 had dissipated, and by 1972.3 most of the flux came from regions greater than about 1 marc s in diameter.

During both epochs of expansion the PA was near  $65^\circ$  whenever a good model could be generated.

Fig. 6 Total flux density of 3C120 at  $\lambda = 3.8$  cm. Points from 1969 to 1971.6 are taken from ref. 12; 1971.6 to 1974.4, ref. 13; 1974.4 to 1975.6, from private communications with W. A. Dent, and the dashed line is interpolated between observations at 2.8 and 4.5 cm reported in ref. 14.





**Table 1** Strong compact extragalactic radio sources

| Source  | Name    | Id | $z$   | $S_{3.8}$ (Jy) | VLBI              | Refs               |
|---------|---------|----|-------|----------------|-------------------|--------------------|
| 0316+41 | 3C84    | G  | 0.018 | 20-58          | slow variable     | 15, 24, 25, 27, 28 |
| 0355+50 | NRAO150 |    |       | 7.3-15         | stable double (?) | 16, 24, 29         |
| 0430+05 | 3C120   | G  | 0.032 | 6.4-15.1       | superluminal      |                    |
| 0923+39 | 4C39.25 | Q  | 0.699 | 8.5-11.8       | stable double     | 5, 24, 29          |
| 1226+02 | 3C273   | Q  | 0.158 | 28-53          | superluminal      |                    |
| 1253-05 | 3C279   | Q  | 0.538 | 11.2-17.5      | superluminal      |                    |
| 1641+39 | 3C345   | Q  | 0.595 | 8.8-11.9       | superluminal      |                    |
| 2134+00 |         | Q  | 1.94  | 11.0-13.4      | slow variable     | 15, 24             |
| 2200+42 | BLLac   | G  | 0.07  | 4.8-14.2       | rapid variable    | 16, 24, 29, 30     |
| 2251+15 | 3C454.3 | Q  | 0.859 | 9.0-20.2       | variable core     | 16, 24, 26, 29     |

Figure 6 shows the total flux density of 3C120 at  $\lambda = 3.8$  cm. This source varies more rapidly and has a greater fractional variation than 3C345 and 3C273. The largest outburst in Fig. 6 begins in mid-1972 and is coincident with the start of the first expansion shown in Fig. 5. The second expansion starts in early 1974 and does not appear to be correlated with any exceptionally large flux event.

### 3C279 ( $z = 0.538$ )

The available data on 3C279 are mainly at  $\lambda = 3.8$  cm, from the period 1970–73 (refs 7, 17–20, 26). Most of this material is from only one baseline, and the interpretations are ambiguous.

The two earliest observations have been interpreted as an expanding double, with separation rate  $\dot{\theta} = 0.27$  mas yr<sup>-1</sup> ( $v/c = 10$ ) (refs 7, 19). The rate is not well determined with only 2 points, but it is consistent with observations at 6 cm in early 1972 (ref. 17), which give the overall size as about 2 mas. A minimum in the 13-cm visibility function on baselines from California to Australia<sup>26</sup> near the beginning of 1970 is also consistent with a simple double model based on the 3.8-cm observations, suggesting that the separation of the two components was similar over that range of wavelengths.

A sequence of observations at 3.8 cm in 1972 (ref. 17) can be interpreted as a new expansion event, since an unresolved weak core was seen in March and April 1972, and this became substantially resolved by November 1972. Another possibility is that all the 1972 data came from a triple source whose overall dimensions were constant, but whose component flux densities varied<sup>17</sup>. The published material is consistent with either model, and further data are necessary to discriminate between them.

### Interpretations

It is of interest to ask what fraction of all sources are superluminal, and whether statistical arguments can be made for or against any of the customary interpretations. These are difficult questions because adequate surveys do not exist at the short centimeter wavelengths, and VLBI surveying is particularly incomplete. However, we can make some comments.

At  $\lambda = 3.8$  cm, 3C345 is the weakest known superluminal source, with mean flux density  $< S_{3.8} \sim 10$  Jy during the expansion. We know of 15 extragalactic sources which have been as strong as 10 Jy at 3.8 cm, at any epoch. Five of these are radio galaxies with most of the flux coming from extended regions. (3C123, 3C353, Vir A, Cen A, Cyg A). The remaining 10 sources are shown in Table 1. The fifth column in Table 1 gives the known range of flux density at  $\lambda = 3.8$  cm. The sixth column gives a comment on the structure seen with VLBI, and the seventh column gives references for those sources not discussed in detail above.

3C84 has several components which vary in flux density but seem to have little motion. NRAO150 has been observed less than the others and its size is less than 0''.001 so that intercontinental observations are necessary for reasonable resolution.

4C39.25 has a well-defined double structure with constant separation, but the total flux density is variable. 2134+004 apparently is more complex than a simple double, and has slow weak variations in its brightness distribution. BL Lacertae has a

double structure which is variable but keeps a constant position angle. The observed changes in BL Lac are not systematic as in the superluminal sources, but it may well be that shorter sampling intervals are required to see them, as this is the most variable source in Table 1. 3C454.3 has a core-halo structure. The variations in total flux density follow those of the unresolved core.

Nearly half the strong compact sources show a superluminal effect. This suggests that mechanisms which require us to be in an especially favourable position are unlikely to be at work. Luminous relativistically moving clouds are one such class, because to see them superluminally we have to be within a small solid angle<sup>1</sup>. However, the blue-shift of the approaching clouds also raises the possibility of a selection effect. Perhaps superluminal sources seem common partly because the blue shift raises their flux density above the minimum level being counted. To study this possibility we need to know the distribution of superluminal sources with flux density, which is unknown at present.

The superluminal sources all show a systematic expansion, and at least one of them shows two epochs of expansion with the same PA. This argues strongly against mechanisms which basically are random and allow both expansions and contractions. Gravitational lenses and some other propagation and opacity effects are of this type. So, too, would be a collection of separated sources which are independent, and flash at random times. Propagation and opacity effects also are unlikely because they predict that the separation would be wavelength dependent, contrary to observation.

The fact that the superluminal effect has been seen in a galaxy as well as in three quasars suggests that it cannot be used as evidence against a cosmological origin for the redshifts.

Thus what is now known can be summarised as follows. Nearly half the strong ( $S_{3.8} \geq 10$  Jy) compact ( $\theta < 0''.01$ ) sources show a superluminal expansion at centimetre wavelengths, and changes in overall size of up to factor 10 in periods of one to two years have been observed.

No systematic contractions have been seen. The separation of components in a source is basically independent of wavelength from 2 to 6 cm, although the components may have different spectra. In 3C273, and perhaps in 3C120, we may have observed an epoch where component evolution produced different sizes at different wavelengths.

The position angle is substantially constant during an expansion. In at least one case (3C120) there have been two distinct epochs of expansion. The position angle is the same for both.

The brightness distribution does not expand uniformly, but there is an overall trend as shown in the graphs. The epoch of zero separation, determined by extrapolation of the trend, may be close to the start of a major outburst in flux density.

The extensive data for 3C345 at 1974.5 and for 3C120 at 1976.2 strongly suggest a structure which can be represented as a simple double, with the two components being approximately equal. 3C273 is more complex. There are both simple (4C39.25) and complex (3C84) sources which do not show

variations in the overall size of the brightness distribution although their total flux densities vary on time scales similar to those for the superluminal sources.

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## articles

# Percolation theory of residual phases in porous media

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*When one fluid displaces another by flow through a porous medium, the fraction of pore space occupied by trapped residual phase correlates with the ratio of viscous to capillary forces in the flow. This dependence, heretofore unexplained, is derived from the mechanics of fluid blobs, the nature of pore structure, and percolation theory. Pore space topology is shown to be as important as geometry in the statistical flow behaviour of large populations of fluid blobs in two-phase flow in porous media. Topology and geometry can each be modelled usefully by a single parameter characteristic of the pore space.*

WHEN porous solids, whether unconsolidated such as powders, granulated solids, soils, and clastic sediments, or cemented and often diagenetically altered, such as bones, sintered solids and sedimentary rocks, have their pore space occupied by two immiscible fluid phases at least two connectivity domains of the non-wetting phase can be observed. In one—the 'continuous regime'—the phase is largely self-connected over the range of many pore diameters, and in the other—the 'blob regime'—the phase exists only as discrete, disconnected 'blobs' surrounded completely by the other phase<sup>1</sup>. The wetting phase, of course, can also exhibit continuous and discontinuous regimes, but these will not be considered here.

The saturation of a phase is defined as the fraction of pore space or void space occupied by that phase. It is observed in flow experiments that when a non-wetting phase in the continuous regime is drained to a saturation below some critical

value (about 0.1–0.5 (ref. 2), depending on the fluids) by the flow of a second, wetting, phase, the former phase breaks up into discrete blobs. In this blob regime, the non-wetting phase is much more difficult to displace from the porous medium. There has previously been no satisfactory theory of these facts, nor of related phenomena of capillary pressure and relative permeability, although some of the elements for the explanation have been available. These constitute blob mechanics and are reviewed and extended here. They are then tied together with new elements from percolation theory. The synthesis makes it plain that the topology and randomness of the pore space are important. In this article, we show how these aspects influence residual saturation.

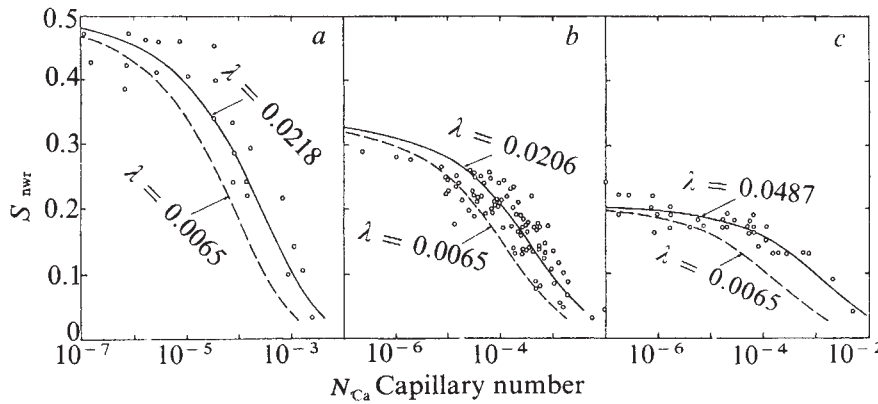
In typical porous media, a fluid at a given saturation in the continuous regime flows with flow rate proportional to the applied pressure gradient (Darcy's Law<sup>1</sup>) even for very low pressure gradients. A fluid phase in the blob regime, however, does not flow at all at a given pressure gradient, if  $\gamma$ , the interfacial tension between the two phases, exceeds a certain critical value.

This contrast is easily explained by reference to the Young–Laplace equation for the pressure jump across an interface between static fluids

$$\Delta p = \gamma \left( \frac{1}{R} + \frac{1}{R'} \right) \quad (1)$$

where  $R$  and  $R'$ , the principal radii of curvature of the interface, are, for fluids in a solid matrix, complicated functions of pore geometry and the contact angle which the interface makes with





**Fig. 1** Residual non-wetting phase saturation as a function of capillary number, experiment and theory. *a*, Foster and McMillen's data,  $\sigma = 2$ ; *b*, Abram's data,  $\sigma = 3$ ; *c*, Lefebvre du Prey's data,  $\sigma = 5$ .

the pore wall. Nevertheless,  $R$  and  $R'$  are of the order of  $D$ , a characteristic pore diameter of the medium; thus

$$\Delta p \sim \frac{\gamma}{D} \quad (2)$$

For typical values of  $\gamma$  (30 dyne  $\text{cm}^{-1}$ ) and  $D$  ( $10^{-4}$  cm)  $\Delta p$  is of the order of  $3 \times 10^5$  dyn  $\text{cm}^{-2}$ , or about a third of an atmosphere. A blob in static equilibrium in a typical porous medium at ordinary values of  $\gamma$  can, therefore, develop large pressure differences across its interfaces. If equation (1) holds at least approximately during slow flow, then with such large pressure differences, disconnected blobs fail to flow because they easily adjust their meniscus configurations to withstand low imposed pressure gradients. According to blob mechanics, the adjustments required are greater the longer the blob is in the flow direction. Hence, longer blobs are more readily mobilised than shorter ones. These predictions have recently been confirmed experimentally for the first time<sup>3</sup>.

Residual saturation should thus also depend on the distribution of blob lengths, which is a consequence of the way in which the continuous regime breaks down into the blob regime. Because this process seems to be governed by randomness of the pore space, we adopt the hypothesis that the size distribution of blobs is that of one material randomly mixed with another within a space which is conveniently approximated as network-like or tree-like. Applied to this hypothesis percolation theory yields the needed size distribution. The resulting predictions of residual saturation agree with extensive experimental data hitherto unexplained.

### Blob mechanics

Imagine a blob trapped in a porous sample surrounded by continuous fluid which is under an imposed average pressure gradient,  $dp/dx$ . In what conditions will this blob be mobilised? To answer this precisely, requires finding the conditions in which the given fixed-volume blob can find no configuration which satisfies the Young-Laplace equation and the boundary conditions imposed by the geometry of the pore space it is in. To identify these conditions approximately, we observe that the viscous flow of continuous fluid around the motionless blob develops a pressure difference,  $\Delta p_{\text{avail}} \simeq l dp/dx$ , across its length,  $l$ , in the flow direction, which is available to drive the blob forward. The pressure difference necessary to dislodge the trapped blob,  $\Delta p_{\text{nec}}$ , is given approximately by  $\Delta p_{\text{nec}} = \alpha\gamma/D$ , where  $\alpha$  is a dimensionless constant which depends on the geometry of the trapping pore space. The blob is mobilised when

$$\Delta p_{\text{avail}} \geq \Delta p_{\text{nec}} \quad (3)$$

and the minimum blob length,  $l^*$ , required for mobilisation, is given by the condition  $\Delta p_{\text{avail}} = \Delta p_{\text{nec}}$ , which can be rewritten as

$$l^* \simeq \alpha\gamma/D \left( \frac{dp}{dx} \right) \quad (4)$$

Now  $dp/dx$  is proportional to the continuous phase fluid velocity,  $v_w$ , according to the extension of Darcy's Law which accounts successfully for two-phase flow<sup>1</sup>

$$v_w = \frac{k_{rw}K}{\mu_w} \left( \frac{dp}{dx} \right) \quad (5)$$

$\mu_w$  is the continuous phase fluid viscosity;  $k_{rw}$  its relative permeability at the specified saturation, and  $K$  the permeability of the porous medium, so that

$$l^* = \left( \frac{\gamma}{v_w \mu_w} \right) \frac{\alpha k_{rw}K}{D} \quad (6)$$

A reasonable approximation which has proved useful for many porous media is<sup>4</sup>

$$D \simeq \beta\sqrt{K\phi} \quad (7)$$

where  $\phi$  is the porosity of the medium and  $\beta$  is a dimensionless constant. Letting  $n^* = l^*/D$ , a dimensionless blob length, gives

$$n^* \simeq \lambda \left( \frac{\gamma}{v_w \mu_w} \right) k_{rw}\phi \quad (8)$$

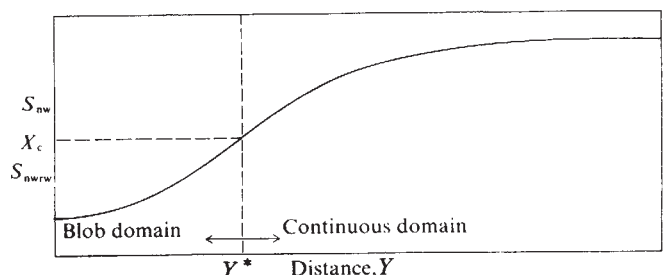
where  $\lambda = \alpha/\beta^2$ . If only continuous phase is flowing,  $v_w = v$ , the superficial velocity, and

$$n^* \simeq \lambda N_{ca}^{-1} k_{rw}\phi \quad (9)$$

where  $N_{ca} = v\mu_w/\gamma$  is a dimensionless ratio of viscous mobilising forces to capillary trapping forces.

An analysis of this sort was also made by Melrose and Brandner<sup>2</sup>, who derived our  $\lambda$  in terms of pore geometry and

**Fig. 2** Schematic saturation profile in a porous medium at a point in time during displacement of a non-wetting phase.



blob configuration. Using estimates which they reported from experiments on unconsolidated packs of uniform, nearly-spherical glass beads or sand grains, we find an approximate value for  $\lambda$  is 0.0065. This microscopic theory of blob mobilisation, following Melrose and Brandner, predicts that for a given blob there is a critical capillary number above which it cannot remain in static equilibrium, a prediction confirmed by experiments with individual blobs<sup>3</sup>.

### Blob population dynamics

The theory of the relationship between the mechanics of individual blobs and the flow behaviour of large populations of them has been missing. Their collective, macroscopic flow behaviour has been investigated experimentally, however. The results show a correlation between the capillary number,  $N_{ca}$ , appearing in equation (9) and  $S_{nwr}$ , the residual non-wetting phase saturation which is irremovable at the given flow conditions no matter how much driving fluid is flushed through the solid matrix. Several investigators<sup>2,5-9</sup> reporting on a large number of experiments using a wide variety of fluids in porous media, have shown that at values of  $N_{ca}$  less than about  $10^{-7}$ ,  $S_{nwr}$  is a constant in the range 0.2–0.5. As  $N_{ca}$  is increased past a value in the range  $10^{-7}$  to  $10^{-6}$  (different investigators report different values)  $S_{nwr}$  begins to decrease. As  $N_{ca}$  is increased further,  $S_{nwr}$  continues to decrease and eventually reaches zero at a value of  $N_{ca}$  between  $10^{-3}$  and  $10^{-2}$ . McMillen and Foster's<sup>8</sup>, Abrams's<sup>5</sup>, and Lefebvre du Prey's<sup>9</sup> experimental data for this capillary number correlation are shown in Fig. 1.

The theory of the relationship between the mechanics of individual blobs and the statistical flow behaviour of large populations of them is supplied, we believe, by percolation theory. We advance here a simple version which predicts the capillary number correlation just described. Before proceeding to it, a brief explanation of percolation theory is needed.

### Percolation theory

Many transport processes can be successfully idealised as transport of an abstract fluid through an abstract medium. Diffusion of a solute through a solvent is an example: the fluid (solute) can be imagined to flow randomly, and the process is termed a diffusion process. If, on the other hand, the fluid flow paths are determined by the medium, but the medium itself is in some sense random, the process is called a percolation process. This classification and the introduction of percolation theory are due to Broadbent and Hammersley<sup>10</sup>.

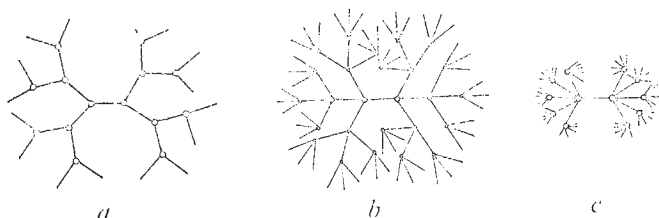


Fig. 3 Examples of Bethe lattices: *a*,  $\sigma = 2$ ; *b*,  $\sigma = 3$ ; *c*,  $\sigma = 5$ .

As a simple example, illustrating those features of percolation theory of relevance here, consider a very large, essentially infinite sheet of non-conducting material divided into identical square cells. At random, we replace a fraction of the cells containing the non-conductor with cells containing a conductor. If the fraction is small, most of the conducting cells occur as monomers—with no conducting cells as nearest neighbours. In particular, there is no continuous path of conducting cells long enough to span the sheet and, therefore, the sheet as a whole is non-conducting. As we increase the fraction of conducting cells, however, dimers, trimers, and larger clusters of connected conducting cells appear, until at some critical fraction, there appears an infinite cluster spanning the sheet. At this critical fraction,  $X_c$ , called the percolation threshold of the medium, the sheet becomes a conductor.

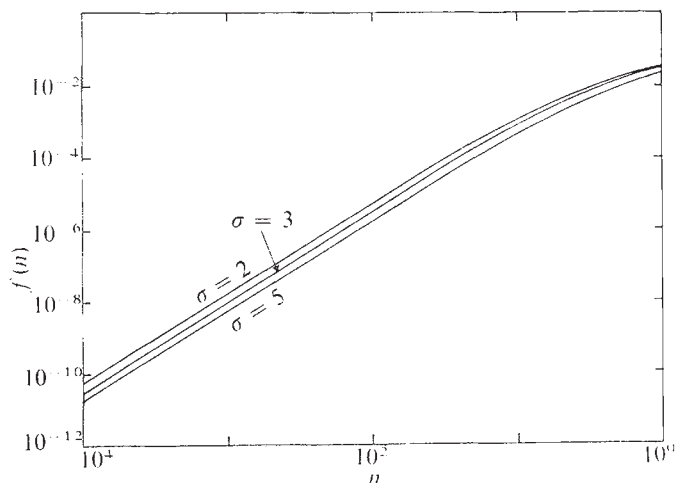


Fig. 4 Cluster size distribution function for a Bethe lattice.

Experiments with large two-dimensional (2-D) square arrays, such as the one described above, reveal an empirical  $X_c$  of 0.5 (ref. 11). That is, at least half the cells must be conducting, if the sheet as a whole is to show a non-zero conductivity. Three-dimensional (3-D) mixtures of conducting silver and non-conducting Bakelite show a conductivity threshold of 30 vol. % ( $X_c = 0.3$ )<sup>12</sup>. Small spheres of nickel mixed randomly with larger spheres of polyvinyl chloride and then compressed together to compaction show  $X_c = 0.10$  (ref. 13). Computer studies of regular networks of chaotically distributed conductors and insulators yield percolation thresholds  $X_c$  ranging from 0.1 to 0.6, depending on the randomness of the distribution and connectivity of the network<sup>11</sup>. A computer-simulated 3-D continuum model gives  $X_c = 0.29$  (ref. 14). Using percolation concepts, equation (9) which holds for an individual blob will now be used to derive a theoretical capillary number correlation.

### Theory of residual phases

Imagine a one-dimensional 'waterflood', a process in which continuous non-wetting phase is being displaced from a porous medium by wetting phase. At a given time, the nonwetting phase saturation profile in the medium at some instant is represented by Fig. 2. At large  $Y$  (distance from position of wetting phase injection) the non-wetting phase is in the continuous regime, but at  $Y = 0$ , the non-wetting phase is in the blob regime, since only wetting phase is flowing. At some intermediate position,  $Y = Y^*$ , the non-wetting phase passes from the continuous to the blob regime. If the flow of non-wetting phase through the medium is such that at a given position  $Y$ , the nonwetting phase is distributed in a manner that leaves it randomly self-connected, then  $Y^*$  is the position where the non-wetting phase saturation,  $S_{nw}$  equals  $X_c$ , the percolation threshold for the void space of the solid matrix; that is, where  $S_{nw}$  is just low enough that the non-wetting phase no longer maintains long range continuity.

According to equation (9), if the capillary number of the flow is vanishingly small, as in very slow flow, then only long—essentially infinitely long—blobs will be mobile; that is, only that non-wetting phase which is in the continuous regime will flow. Thus, saturation must exceed  $S_{nw} = X_c$  for non-wetting phase to flow if  $N_{ca}$  is very small. Let waterflood residual non-wetting phase saturation be defined as

$$S_{nwrw} \equiv \lim_{N_{ca} \rightarrow 0} S_{nwr}(N_{ca})$$

then  $S_{nwrw} = X_c$ . Experimentally, as  $N_{ca}$  becomes small,  $S_{nwr}$  asymptotically approaches  $S_{nwrw}$ , whose value typically lies between 0.2 and 0.5, as compared with a value of  $X_c$  of 0.25–0.30 for a completely random 3-D medium.

If, however,  $N_{ca}$  is not infinitesimally small, then all blobs



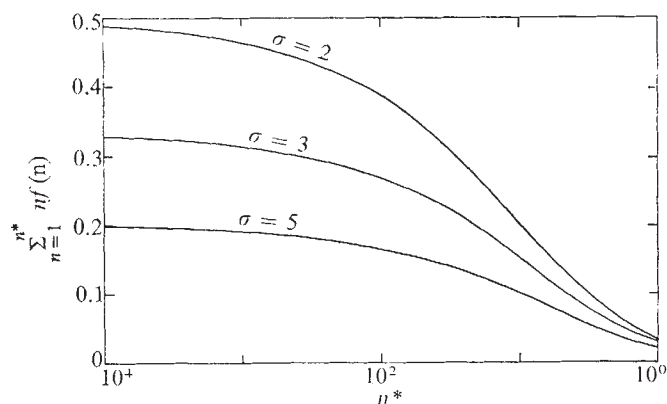


Fig. 5 Partial sum of the Bethe lattice cluster size distribution function.

of non-wetting phase longer than  $n^*$ , as given by equation (9), will flow, blobs shorter than  $n^*$  being trapped. When  $S_{nwr}$  decreases through  $S_{nwr} = X_c$ , the non-wetting phase breaks up into discontinuous blobs. Imagine that the distribution of projected lengths of these blobs is given; call it  $f(n)$ . That is,  $f(n)$  is the expected number of blobs of length  $n$  in a large porous sample, divided by the total number of pores in the sample. The fraction of pore volume occupied by trapped blobs of length  $n$  is approximately  $nf(n)$ . (Here we ignore the difference between the number of pore bodies a blob occupies and its projected length, in pore diameters, along the direction of the pressure gradient.) On average, the volume of non-wetting phase trapped per pore volume, which is the residual non-wetting phase saturation, at a given  $N_{Ca}$  is

$$S_{nwr} = \sum_{n=1}^{n^*} nf(n) \quad (10)$$

where  $n^* = \lambda N_{Ca}^{-1} k_{rw} \phi$ , as at (9).

If  $f(n)$  were available a theoretical  $S_{nwr}$  ( $N_{Ca}$ ) could be calculated from equations (9) and (10). If the already mentioned hypothesis is correct and the non-wetting phase is distributed in such a manner that it is randomly self-connected, then  $f(n)$  may be obtained from a cluster size distribution for a 3-D random medium, a 2-D analogue of which was described above. A convenient approximation to such a medium, for which an analytical expression for the cluster size distribution is available, is the Bethe tree, or Bethe lattice. A Bethe lattice is a collection of bonds and sites,  $\sigma + 1$  bonds per site, illustrated in Fig. 3 for  $\sigma = 2, 3$ , and 5. Let a fraction,  $X$ , of the bonds be conducting, the balance being non-conducting. Fischer and Essam<sup>15</sup> give an analytic expression for the size distribution of conductive clusters in a Bethe lattice; the distribution depends on  $\sigma$  and  $X$ . They find that the percolation threshold is at  $X = X_c = 1/\sigma$ . In Fig. 1, the values of  $S_{nwr}$ , and hence of  $X_c$ , can be seen to be about 0.5, 0.33, and 0.2, respectively, for Abrams' Berea sandstone, Foster and McMillen's Dalton sandstone, and Lefebvre du Prey's sintered teflon porous samples.

To model the topology of these porous media by Bethe lattices, one should take  $\sigma = 2, 3$ , and 5, respectively, for the three media. The value of  $\sigma$  chosen to fit the value of  $S_{nwr}$  for a given porous medium is, in theory, a measure of the connectedness of the pore space of that medium. Higher values of  $\sigma$  indicate more inter-pore connections per pore, and thus a larger number of pathways one or more of which can be conductive to non-wetting phase.

With  $X = X_c = 1/\sigma$ ,  $f(n)$  can be calculated from the analytic expression for Bethe trees in which  $\sigma = 2, 3$ , and 5, respectively, with results shown in Fig. 4. Taking partial sums to get  $S_{nwr}$  as prescribed by equation (10) gives the results shown in Fig. 5.

For his Dalton sandstone sample, Abrams<sup>8</sup> obtained for the relative permeability  $k_{rw}$  needed in equation (9), the experi-

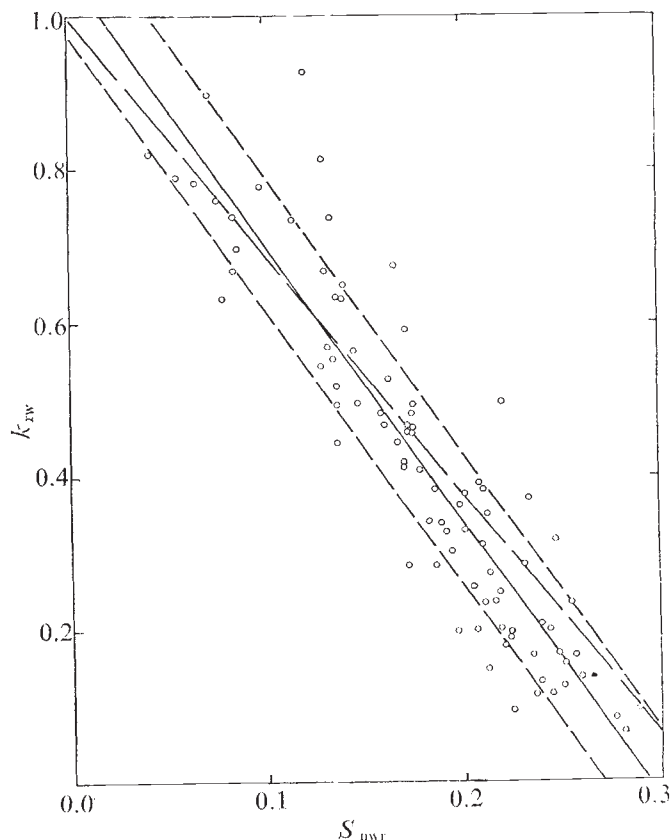
mental dependence on  $S_{nwr}$  shown in Fig. 6. This dependence is nearly linear over most of the range of  $S_{nwr}$ . By definition  $k_{rw} = 1$  at  $S_{nwr} = 0$ , and experimentally  $k_{rw} = 0.06$  at  $S_{nwr} = S_{nwrw}$  for Abrams' Dalton sandstone sample. A straight line between these two endpoints falls within a standard deviation of the least squares linear fit to the data (Fig. 6). Lacking the complete relation between  $k_{rw}$  and  $S_{nwr}$  for the other two porous media, we take as an estimate for all three media a straight line through the endpoints, the endpoint at  $S_{nwr} = S_{nwrw}$  being provided by experiment for each porous medium.

For a given residual non-wetting phase saturation  $S_{nwr}$ ,  $n^*$  can be found from  $f(n)$  and equation (10), that is from Fig. 5;  $k_{rw}(S_{nwr})$  can be found from the endpoint linear estimate;  $\phi$  from experiment; and thus  $N_{Ca}$  can be calculated from equation (9) for a specified value of  $\lambda$ . There are two parameters: besides  $\lambda$ , which represents the relevant geometry of the pore space (introduced before equation (4) and at equation (7), there is behind  $f(n)$  the topological parameter  $\sigma$  which we take as 2, 3 and 5, respectively, for the three porous media in Fig. 1. For  $\lambda$  we have tested various numbers: the value of 0.0065 based on Melrose and Brandner's estimates; and best-fit values of 0.0218 for Foster and McMillen's data, 0.0206 for Abrams' data, and 0.0487 for Lefebvre du Prey's data. The theoretical relations are plotted as  $S_{nwr}$  against  $N_{Ca}$  in Fig. 1a-c. The fitted  $\lambda$ 's for the three consolidated porous media considered are consistently higher than the 0.0065 estimate for unconsolidated porous media. The difference is probably significant despite the uncertainties in Melrose and Brandner's estimates and the simplifying assumptions of the percolation theory employed here.

## Conclusions

The relationship between residual non-wetting phase saturation and the capillary number of wetting phase flow in Fig. 1 is

Fig. 6 Relative permeability as a function of residual non-wetting phase saturation for Abram's Dalton sandstone sample. —, Least squares linear fit; ---,  $\pm$  one standard deviation; - - -, endpoint linear approximation used in the theory.



successfully predicted from the mechanics of blob mobilisation and the percolation theory of transport. Central is a percolation concept of the size distribution of conductive clusters when conducting bonds are randomly located in a lattice. The clusters model the blobs of residual phase. Their size distribution depends on the connectivity properties of the lattice. These properties model the topology of the pore space. Geometry of the pore space dictates the relation between the size of a blob and critical flow conditions for mobilisation of that blob. A practical implication of the theory is to reduce the problem of two-phase flow in porous media in first approximation to determining two parameters. One, the scale factor  $\lambda$  in this report, characterises the pore-geometry-dependent resistance to passage of a meniscus separating the phases. The other, the connectedness measure  $\sigma$  here, characterises the pore-topology-dependent number of alternate routes of meniscus passage. Concepts from percolation theory also lead to successful

explanations of capillary pressure and relative permeability data from porous media<sup>16,17</sup>.

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# An 8,000-yr palaeoclimatic record of the 'Double-Hale' 45-yr solar cycle

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*A series of isostatically-emerged Holocene beach ridges on the east side of Hudson Bay have been precisely surveyed and dated back to 8,300 BP. They number 185 and show a nearly uniform rhythm of about 45 yr correlated here with the 'Double-Hale' solar magnetic cycle. (Time is in sidereal yr.) Longer correlations are suggested with eustatic sea-level curve and with planetary conjunction cycles. The coupling mechanism with terrestrial meteorological behaviour is believed to be by means of stratospheric generation of polar cirrus cloud and low-latitude ozone, affecting the planetary albedo.*

A CONTINUOUS sequence of 185 Holocene, radiocarbon-dated raised beach lines on the eastern side of Hudson Bay, Canada, have a  $45 \pm 5$ -yr cyclicity that reflects storminess and high tide crescendos (Fig. 1). The strandlines have been precisely measured by theodolite surveys and followed by helicopter and air photograph studies along the coast. They can be observed as far as Ungava Bay (Hillaire-Marcel). They have been known since the seventeenth century but have only now been surveyed<sup>12</sup>. The beaches have been dated by radiocarbon analyses back from the present to 8,300 yr BP (sidereal) when the 'Tyrrell Sea' began to appear as a result of deglaciation during the Flandrian transgression. The relative uniformity of the 185 emerged beaches suggests a regular dynamic mechanism. Thus  $8,300/185 = 44.86$ , conveniently rounded to 45. If the beach ages are plotted at 45-yr intervals, they are found to correspond closely to the radiocarbon dates.

A complicating factor is introduced by the unfortunate mid-Holocene departures of the radiocarbon chronology from sidereal time which tend to distort those ages to lower values, but these systematic errors are in part counteracted by comparable errors introduced by old carbon from freshwater dilution.

The uniformity of the  $45 \pm 5$ -yr cyclicity is implied by the mean uniformity of beach widths, but their heights become progressively greater as one goes back in time because of an exponentially decaying isostatic curve. The present rate of uplift is  $1.1 \text{ cm yr}^{-1}$ , but around 7,000 to 8,000 BP it was ranging

through  $5\text{--}10 \text{ cm yr}^{-1}$ , and is thus comparable with the behavior of the Baltic Shield in the Gulf of Bothnia<sup>4</sup>.

The beaches are so sharp and clearly preserved due to a happy coincidence of genesis and history. Their materials were partly eroded and redeposited in ice-foot conditions, which is a much more energetic process than normal long-shore drift. Further, the continuous isostatic uplift rapidly takes each beach up above the reach of storm waves and wind-driven ice floes, so that they are preserved in their pristine state. The climate maintains a continuous permafrost beneath a sparse cover of arctic tundra vegetation. There is no human interference with their natural state.

A strandline of this sort is preserved in the topography only at certain moments in time, when, for example,  $dI + dE \rightarrow 0$  where  $dI$  is isostatic uplift over a given interval,  $dt$ ; and where  $dE$  is eustatic rise. When they are both in phase, and moving at the same rate, the shoreline will remain constant long enough for a recognisable beach to form. A second mechanism for beachridge formation is during cycles of increased storminess when seasonal onshore winds briefly imitate the effect of a eustatic rise.

Inasmuch as the isostatic factor has a very sluggish rate of velocity change (maximum  $1 \text{ cm yr}^{-1}$  in 100 yr), compared with eustatic factor ( $\approx 1 \text{ cm yr}^{-1}$  in 1–10 yr), any systematic differences between the isostatic uplift curve and the actual height of a given series of beach ridges must reflect an anomalous eustatic oscillation.

By constructing a glacioisostatic crustal recovery curve and carefully measuring the anomalous high and low series of beach levels, it was, therefore, possible to distinguish a eustatic factor from the isostatic, in the manner already demonstrated for Hudson Bay by Andrews<sup>5</sup>. Some of the early beaches are today uplifted to a maximum of 315 m.

Comparison of the eustatic residual with earlier versions of the Holocene eustatic curve obtained from low-latitude situations<sup>5</sup> shows an interesting degree of coincidence. It is clear that sudden drops or rise of sea level of the order of 2–5 m are worldwide in effect and may occur within time spans of 100–200 yr ( $dE = 1\text{--}5 \text{ cm yr}^{-1}$ ). These negative sea-level oscillations cor-





**Fig. 1** Typical series of isostatically raised strandlines situated in Richmond Gulf on the eastern side of Hudson Bay, Canada. The sand-gravel beaches are well-preserved by permafrost and recur with a great regularity at around every 45 yr, representing cycles of storminess. A higher ridge, like that in the right foreground, represents a modification introduced by the eustatic factor, in this case a sharp regression followed by a rise, but not enough to overcome the isostatic emergence rate.

relate with documented climatic cooling and are assumed to be glacioeustatic (Fig. 2).

This fact can have a profound significance on climate prediction. If  $dE = 1 \text{ cm yr}^{-1}$ , the equivalent ice volume is  $4 \times 10^3 \text{ km}^3$ . The record shows that several cold spells sufficient to generate  $1-2 \times 10^6 \text{ km}^3$  of ice within one or two centuries have actually happened within the recent history of man in the present (Holocene) interglacial climatic conditions. The total ice volume of Greenland today is less than  $2.6 \times 10^6 \text{ km}^3$ , equivalent to  $dE \approx 6 \text{ m}$ . Approximately 20 cold spells, each sufficient to generate at least  $1 \times 10^6 \text{ km}^3$  of excess glacier ice (above the global equilibrium level), have occurred during the last 6,000 yr, thus about one per 300 yr. No obviously predictable cyclicity is apparent.

### The 45-yr cycle of storminess

The normal climate of the Hudson Bay is continental arctic, commonly rather calm and mild in summer and frigid in winter. Westerly storm tracks usually pass to the south. When, however, they periodically reach the latitude of Hudson Bay considerable wave action leads to rapid longshore drift and beach-building. The beach ridges were first attributed to these storms by Captain L. Foxe in 1631.

Because of the steady isostatic uplift (over  $1 \text{ cm yr}^{-1}$ ) it is unlikely that after a 45-yr interval a new cycle of storms will cause the younger beach accumulation to overwhelm the earlier one. Thus the 185 beach lines preserved seem to represent a true record of somewhat regularly spaced meteorologic events. These are modulated, with larger and smaller beaches, by the glacio-eustatic factor mentioned above. A comparable but non-uplifted sequence of beach ridges on the Alaskan Arctic coast is attributed to meteorologic pulses related chronologically to the sunspot cycle<sup>7</sup>.

Such a regular succession of climatically related geomorphic phenomena cannot be easily explained away by any steady state or stochastic hypothesis and invite a search for an exogenetic (cosmic) cause. The solar cycle seems to be the most obvious candidate. In recent years, it has been demonstrated with a reasonable degree of certainty that the sunspots are in some way expressions of solar climate that is tidally induced by planetary alignments<sup>8</sup>. If this is correct, two problems have to be considered: first, which of the planetary conjunctions or other configurations seem to have the leading role? And secondly, what is the mechanism of energy transfer from solar tidal behaviour to terrestrial climate?

If the second question is not met in some way the first does not become irrelevant, but in order to test the probability of the second, the timing of the first-named problem can possibly provide some guidance.

### Solar tides

The timing of the basic '11-year cycle' is known to fluctuate between about 9 and 13 yr, but it is statistically around 11.1–11.3, thus close to that of the jovian yr, 11.8 sidereal yr. Equally, or more important, are the periods of other smaller but closer gravitational bodies affecting the Sun, notably Venus, Mercury and the Earth–Moon pair<sup>8</sup>. As with oceanic tides the problem of solar tides is quite complex.

The three-century record of closely observed sunspot behaviour is highly informative. Clearly the 22-yr heliomagnetic or Hale cycle is perhaps more significant than the familiar 11-yr pattern, and a study of the peak sunspot numbers (maxima) discloses a fourfold repetition of the 11-yr sequence, usually within a progressive rise to a crescendo every 45 yr (that is 40–49 yr, the so-called Double Hale cycle). Turning points (minima) were 1699, 1744, 1784, 1833, 1877, 1923, 1964 (ref. 9). A multiple (the King–Hele cycle) around 178–180 yr, gives the turning points in the long term sunspot series.

If one considers the less precise but historically quite well documented sunspot record of the Chinese<sup>10</sup> it becomes clear that there were lengthy periods with few sunspots (those observable without telescopes). The great sunspot dearth of the middle ages is particularly well known. In the record of planetary motions, the year AD 1433 is particularly significant, being the zero check year: with all planets in conjunction<sup>11</sup>. It is thus feasible to mark out various longer cycles from this date in order to see what terrestrial phenomena, if any, seem to match them<sup>12</sup>. The next significant planetary conjunction should be AD 1982.

### Sidereal and radiocarbon years

Care should be taken to remember that the astronomical conjunctions are usually listed in terms of sidereal years, whereas much of the Holocene geological record is documented in radiocarbon years, which as mentioned earlier, have variable values. While at the beginning of the Holocene (10,000 yr BP), according to the varve-record, the departure was probably only of the order of  $\pm 200$  yr, the middle Holocene was marked by errors in the radiocarbon age of 800 or even 1,000 yr, appeared too young<sup>13,14</sup>.

The problem has to do with solar wind interacting with the earth's magnetic field which, as Bucha<sup>15</sup> has shown, has been weakening within the period of precision observation. This causes fewer cosmic rays to be deflected and thus more radiocarbon is generated. Accurately counted tree-ring samples indicate that between 200 and 700 BP there was a  $^{14}\text{C}$  flux up to 2% above normal and thus dates in this period are up to 60 yr too young. Back to about 3,000 BP there were minor fluctuations, with some dates too old, but during the mid-Holocene there was a systematic departure giving dates too young by up to 800 yr (around 6,500 BP). The short term departures can also cause problems, because statistical peaks in site dates<sup>15</sup> may be artificially amplified; while the groupings almost certainly have a geomorphic-climatogenic cause, the possibility of  $^{14}\text{C}$  flux variations should always be tested using tree-ring or varve standards, if possible.

An interrelationship between geomorphic-climatogenic events (such as eustasy, glaciation) and terrestrial magnetism is proposed by Olausen and Svenonius<sup>17</sup>. It is significant that they correlate ice-loading and water-load distribution with reversals and predicted a magnetic reversal for some stage during the Flandrian transgression. A Gothenburg Event around 13,750–12,350 BP has, in fact, been widely recorded<sup>18,19</sup>. It could be expected that briefly during such a reversal there would be a regular deluge of radiocarbon over the whole Earth that for a few centuries might introduce seriously young dates.

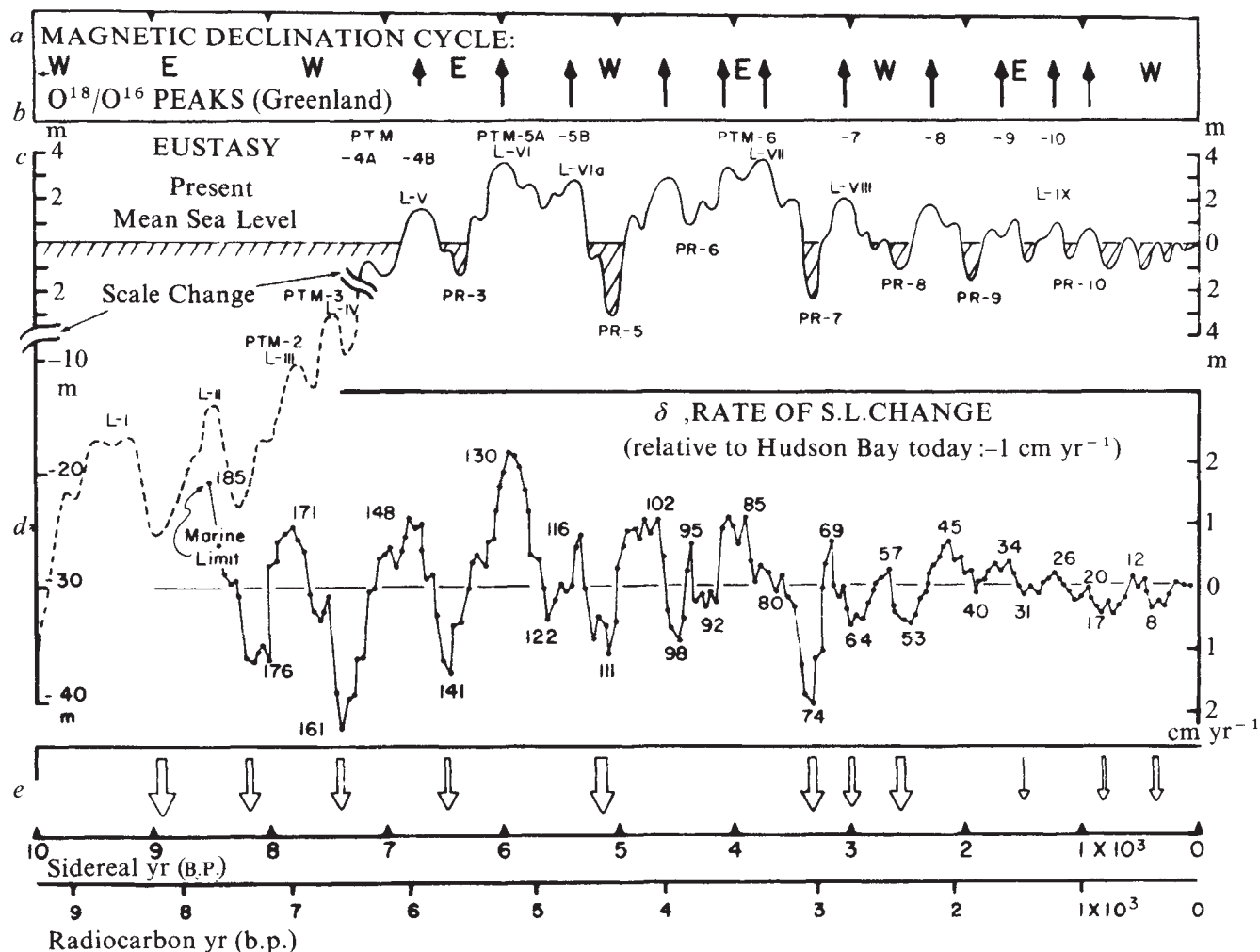


Fig. 2 Five records of 10,000 yr Holocene climate indicators compared. *a*, Magnetic declination cycle<sup>33</sup>: times of E-W inflections seem to represent times of lowest latitude (continental) sites for the magnetic pole, the present site being close to Hudson Bay, while the intermediate passage is over the ocean. (Question: does this have a climatic triggering effect?). *b*, The  $^{18}\text{O}/^{16}\text{O}$  isotope peaks, as indicated by the Camp Century ice core from Greenland<sup>36</sup>.  $^{18}\text{O}$  increases correspond to warmer fluctuations and to highest eustatic sea levels in the tropical oceans<sup>36</sup>. (Note: although there has been criticism and revisions of this record, the younger part shown here is not affected by the modifications and is apparently consistent for our own data shown below). *c*, Eustasy. This curve represents a slight updating of the Fairbridge Curve of 1961<sup>30</sup> and 1976<sup>6</sup>, adjusted to sidereal time. Essentially, it represents a statistical summation of geomorphic sea-level traces (beach ridges, inter-tidal facies, coral in growth position) dated by radiocarbon. No adjustments are made for postglacial hydro-isostatic loading effects. PTM 1/10 peaks are based on Mörner's<sup>36,37</sup> Postglacial Transgression Maxima in S.W. Sweden. L-I/IX are Tooley's<sup>38</sup> Lytham raised beach levels from N.W. England. A comparable but younger series from New Zealand<sup>39</sup>, and numerous other compatible records (Ters, Greensmith, Berglund, Martinsson), are omitted to avoid clutter. PR 1/10 are Mörner's Postglacial Regression Maxima. *d*, Delta,  $E$ , the rate of sea level change, as determined by Hillaire-Marcel<sup>11,2</sup> relative to the present-day east coast of Hudson Bay. Here the approximate crustal rise today is  $1\text{ cm yr}^{-1}$  and the record, with the isostatic variability removed, now indicates rates of sea-level rise and fall. The inflection points (of no change) are numbered according to a continuous set of 185 numbered beach ridges on Hudson Bay, dating back 8,300 yr (sidereal). The relatively uniform geomorphic spacing of the beach ridges suggests a cyclicity of  $\sim 45\text{ yr}$  in storminess on Hudson Bay. *e*, Glacial advances. Major advances of mountain glaciers are known from radiocarbon dating of distal moraine belts, as recently summarised by Denton and Karlen<sup>40</sup> and others. Material is taken mainly from Alaska, Greenland, Scandinavia (Lapland), Patagonia and New Zealand: main advances occur simultaneously in both hemispheres. An almost identical set of dates was independently obtained at the same time from the Alpine glaciers by Patzelt<sup>41</sup>. Size of arrow is scaled to reflect magnitude of advance.

## Little ice ages

It is not suggested that a regularly recurring minor planetary event of this sort can initiate an ice age condition. On the contrary, even the massive effects of the Milankovitch precession-tilt-orbital, now widely accepted<sup>20-22</sup>, cannot generate glaciation phases on an earth that is not palaeogeographically prepared, which requires a blocked oceanic circulation and an asymmetric distribution of major continental land areas in one hemisphere, to create climatically sensitive latitudes. Only 10% of all geological time has thus been favourable to ice ages, and within an ice age of perhaps  $2\text{--}10 \times 10^6\text{ yr}$  only 10% of that time is occupied by runaway glacial advances of continental dimensions (Pleniglacials). The rest of the time is filled by interglacial or stadial/interstadial climates. Nevertheless, it would only take a minor temperature departure for a few centuries in

the north of Britain to trigger another glacial stage in the present sensitive state of global climatic instability. What is needed to explain little ice ages of a few centuries or less is, therefore, merely a small climatic pulse.

The critical year AD 1433 was first brought to attention by Pettersson<sup>23</sup> who believed that it correlated with catastrophic tides and flooding. In fact, the oceanic tidal effect of planetary conjunctions is quite minor, but the solar tidal response may have been quite different. This AD 1433 is the all-planetary conjunction, the so-called zero-check year. From Schöve's long term Chinese sunspot evidence and other data, the year 1433 coincided by a remarkable high but short-lived period of sunspot activity, and after a brief but dramatic rise (to the  $> 150$  spot level) there was a second sunspot dearth (Maunder Minimum) at the same time attended by a major rise in  $^{14}\text{C}$



flux (ref. 25 and P. E. Damon). Historical climatic analyses<sup>26-28</sup> show that 1433 initiated a time of great instability, culminating in the great medieval little ice age, with its attendant human suffering. It seems that the conjunction time coincided with a high sunspot state, which immediately followed by a low-spot condition indicates a minor glacial event.

Accordingly, taking AD 1433 as the base year, the major planet conjunctions have been traced back through the Holocene. Several years ago, a first attempt at this was made by Stacey<sup>11</sup>, but at that time it was limited by interpretations and errors in the radiocarbon record. He attempted to correlate the planetary periods with the Fairbridge eustatic curve, assuming the fluctuations of the latter to represent (inversely) the rise and fall of glaciation. What has come out from the more recent studies of the Holocene eustatic record<sup>30,31</sup> is that the negative fluctuations that correlate with glacier advances and little ice age conditions are very brief events within a rather mild interglacial climatic pattern. The negative sea-level fluctuations are of the order of 3-5 m. In the last 8,000 yr there have been seven of these major departures; they show up equally in the tropical eustatic curve, in the Hudson Bay beach ridge curve and in numbers of glaciological and marine sedimentological curves. They are rather evenly spaced and despite difficulties in establishing their sidereal dates mentioned above, their regularity is striking. It was therefore considered that the seven recurrences of the 1,134-yr planetary conjunction stadial cycle should be seriously considered as being involved in providing a trigger mechanism.

With the reservations mentioned earlier concerning radiocarbon chronologies (apart from geological field errors) we would, nevertheless, like to draw attention to both the 567-yr and 1,134-yr astronomical cycles (major planets in conjunction). Going back from AD 1433 (= 517 BP), the turning points on the 1,134-yr cycle were 1,651, 2,785, 3,919, 5,053, 6,187 and 7,321 BP (sidereal) years. The hemicycle years (the 567-yr sequence) were 1,084, 2,218, 3,352, 4,486, 5,620, 6,754 and 7,888 BP.

Equally important in all probability is the solar activity tidal cycle (King-Hele) of 178-179 years. The Camp Century (Greenland) ice core showed both this and the 80-yr Gleissberg Cycle. It appears in the major Hudson Bay anomalies and various other records.

Certain coincidences are rather compelling: the great mid-Holocene glacial readvance (known in all glaciated regions), and identified in world oceans with a 6-m drop in sea level (PR-5 of the Baltic and beach 111 of Hudson Bay), coincides precisely with the fourth cycle back from AD 1433, that is, at

5,053 BP. It also matches the 25th cycle back of the 178-180-yr King-Hele cycle. Other dates are less than compelling. At present, we can only say the results look interesting.

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## The size of Rous sarcoma virus mRNAs active in cell-free translation

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*Sedimentation analysis of mRNA from RSV-infected chick cells suggests that Pr76, the precursor to the virus structural proteins, is synthesised on a mRNA of similar size to virion RNA, whereas a protein with molecular weight 70,000 tentatively identified as the non-glycosylated precursor to the viral glycoprotein, gp85, is synthesised on a smaller size class of mRNA.*

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Rous sarcoma virus (RSV) contains a genomic virion 70S RNA which in denaturing conditions dissociates into two apparently identical 30-40S RNA subunits<sup>1-5</sup>. We and others have translated RSV virion 30-40S RNA in cell-free systems derived from mammalian cells<sup>6,7</sup>. The major *in vitro* product of RSV virion 30-40S RNA is a polypeptide similar, if not identical, to the 76,000 molecular weight precursor to the group-specific (gs) antigens, Pr76, which is found in RSV-infected cells<sup>6-8</sup>. Pr76 synthesised *in vitro* in response to RSV virion 30-40S RNA can be labelled with *N*-formyl-methionine derived from the initiator tRNA, fMet-tRNA<sub>F</sub>. Since fMet can

only be incorporated at the N terminus, this result indicates that the N-terminal end of Pr76 arises directly from an initiation event and not as a result of cleavage of a larger molecule. Since all other active initiation sites on eukaryotic mRNAs seem to be near the 5' end of mRNA molecules, we interpret this result to mean that the gene for the gs antigens is located at the 5' end of the viral RNA (ref. 6).

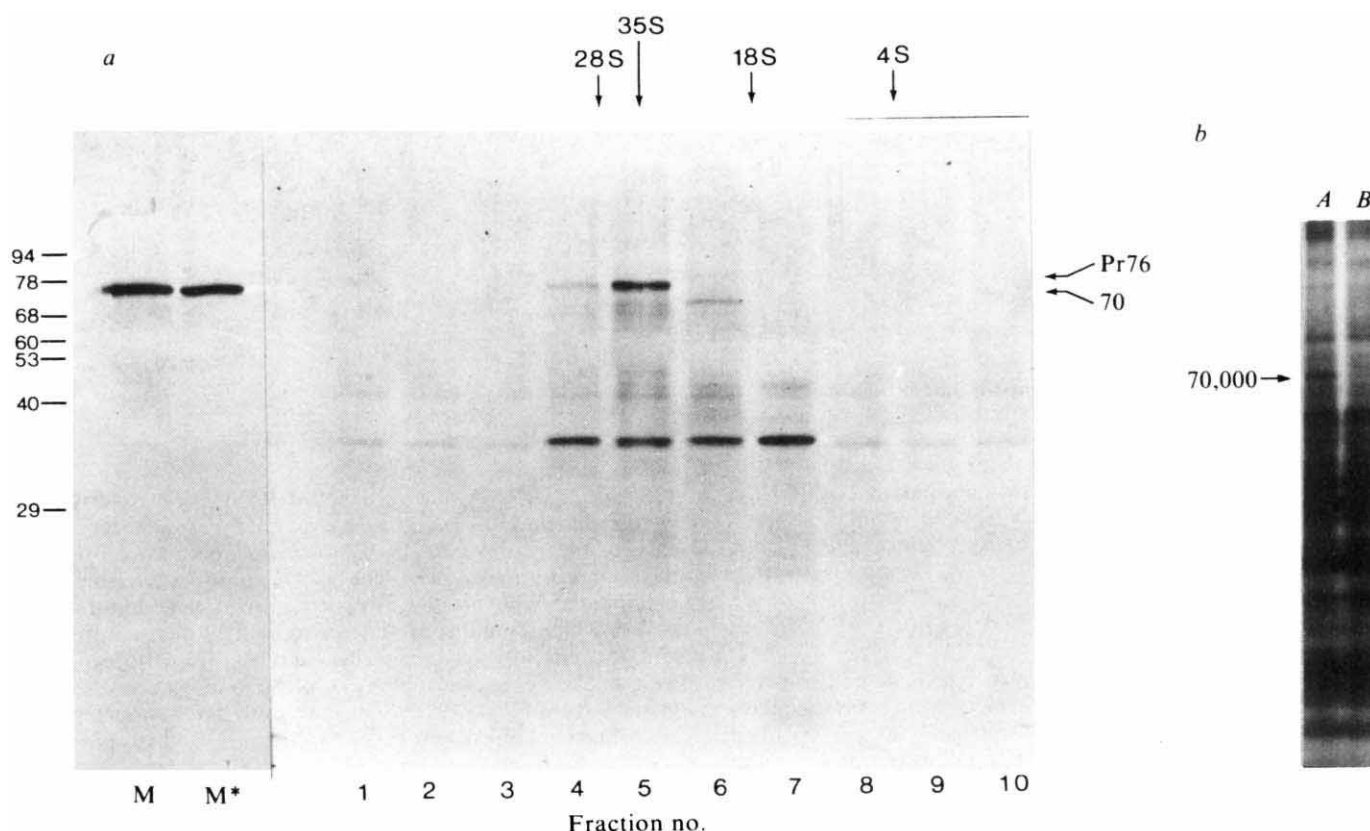
In addition to the gene for the gs antigens, the *gag* gene, RSV virion RNA contains genes for the viral reverse transcriptase, the *pol* gene<sup>9</sup>; the viral envelope proteins, the *env* gene<sup>1,5</sup>; and a gene coding for cell transformation, the *src* gene<sup>1,4,5</sup>. The *src* gene has been positioned at the 3' end of the 30–40S RNA adjacent to the 3' poly(A) tail, and the *env* gene to the 5' side of the *src* gene<sup>1,4,5</sup>. Whether the *src* gene encodes a protein is not yet known.

All the virus-specific RNA present in RSV-infected cells has the same sense as the virion RNA (ref. 10), and two size classes of viral RNA, both of which are present in polyribosomes, have been found in the cytoplasm of RNA tumour virus-infected cells. These are 30–40S virion-like RNA, and a smaller RNA species described by several authors as desimenting between 14S and 27S, the precise size estimate varying with the virus examined and the techniques used<sup>11–15</sup>.

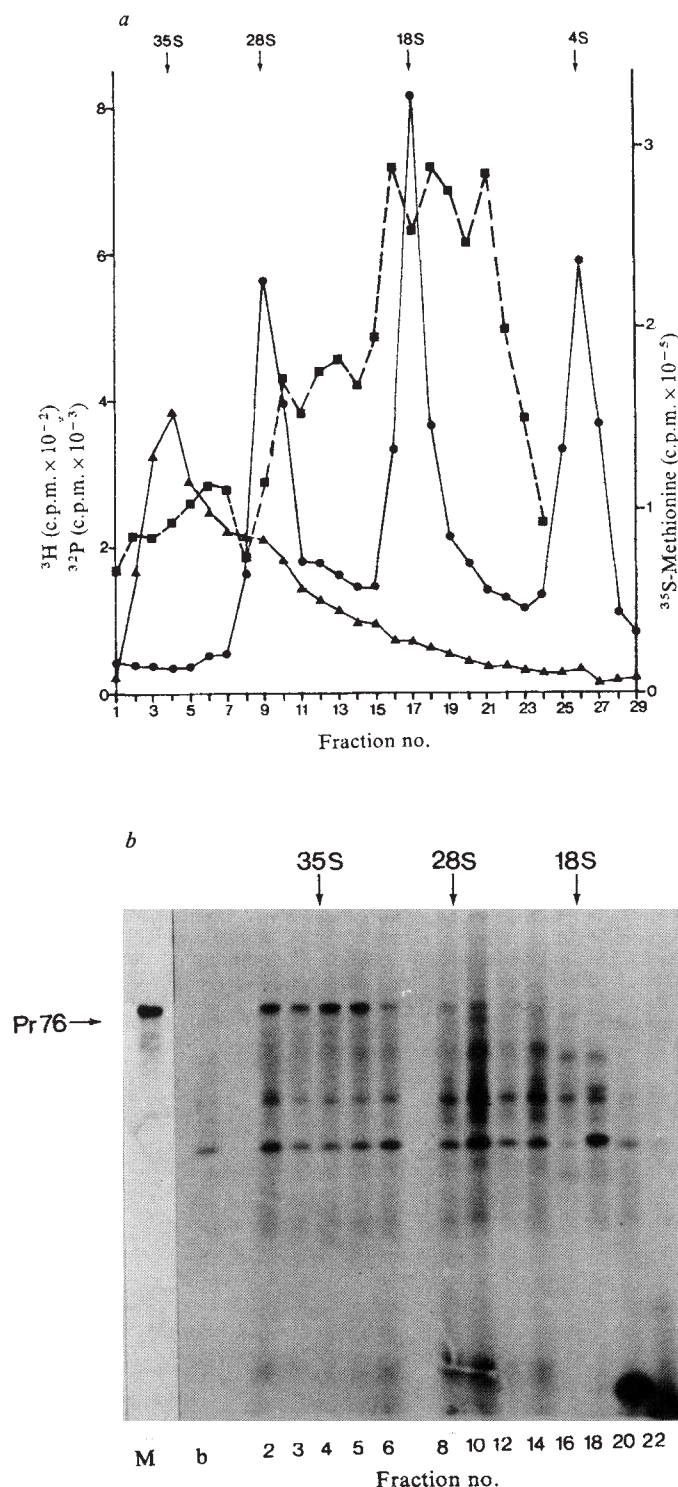
These observations have led to various models for RNA tumour virus protein synthesis<sup>6</sup>. At one extreme is the suggestion that 30–40S RNA is the only viral mRNA, and that the viral proteins are synthesised as a single polypeptide precursor which is subsequently cleaved. At the other extreme is a model in which each viral gene is transcribed into a separate mRNA species. The first model would predict that equimolar amounts of each of the viral proteins are synthesised in infected cells, and would consider the sub-genomic viral RNAs and the failure to make anything other than Pr76 *in vitro* in response to 30–40S virion RNA as experimental artefacts. By contrast the second model predicts that since each mRNA may be present in a different amount and translated at a different efficiency, the viral proteins need not be synthesised in equimolar amounts. This model also suggests that virion 30–40S RNA has internal initiation sites and that they are inactive.

To examine the mechanism by which RNA tumour virus proteins are synthesised, we have isolated total poly(A)-containing cytoplasmic mRNA from RSV-infected chick cells. This mRNA has been fractionated on denaturing sucrose gradients, translated in mammalian cell-free systems derived from mouse ascites and L cells. Because infection by RNA tumour viruses does not shut off host-cell metabolism and viral

**Fig. 1** *a*, SDS-polyacrylamide gel electrophoresis of viral polypeptides synthesised in the ascites cell-free system in response to fractionated RSV-infected chick embryo fibroblast mRNA and RSV virion RNA. Total poly(A)-containing RNA (mRNA) was isolated from *c/o* chick embryo fibroblasts infected with cloned nd RSV using methods described previously<sup>8,16</sup>. mRNA (25 µg) and <sup>32</sup>P-labelled whole mouse embryo non-poly(A)-containing cytoplasmic RNAs were fractionated separately on two 4.5-ml 2–10% sucrose gradients containing 85% formamide<sup>17</sup>. RNA samples were heated at 37 °C for 5 min in 85% formamide buffer before centrifugation for 16 h at 40,000 r.p.m. in a Beckman SW56 rotor at 25 °C. Gradients were fractionated and 30 µg carrier ascites cell tRNA was added to each fraction of the mRNA gradient. The RNA was precipitated after addition of 0.2 M NaCl and 2.5 vol ethanol, reprecipitated twice, washed once in 95% ethanol and dissolved in water. RSV virion 30–40S RNA was prepared as described<sup>6</sup>. RNA from the gradient fractions 1–10 and virion RNA (M) were translated in the mouse ascites cell-free system<sup>6</sup> and 20 µl samples of the cell-free products immunoprecipitated using the double antibody method<sup>6</sup> with antiserum raised against whole disrupted AMV. The products made in response to virion RNA were also immunoprecipitated with antiserum raised against AMV p12 (M\*). The immunoprecipitates were analysed by electrophoresis on a single 15% SDS-polyacrylamide slab gel and the gel prepared for fluorography as described<sup>18</sup>. Because the counts incorporated in response to virion RNA were greater than with virion RNA, the exposure time of tracks M and M\* was only 8 h whereas tracks 1–10 were exposed for 2 weeks. The positions of the marker RNAs run in the parallel gradient are indicated. *b*, SDS-polyacrylamide gel electrophoresis following immunoprecipitation with anti-AMV gp85 serum of polypeptides synthesised in the ascites cell-free system in response to 20–25S RNA from RSV-infected chick cells (*A*) and uninfected duck cell (*B*). mRNA was isolated from infected chick embryo fibroblasts and uninfected duck cells and fractionated on 85% formamide sucrose gradients as for *a*. RNA (referred to as 20S–25S RNA) sedimenting between the positions of the 18S and 28S rRNA markers run in a parallel gradient was pooled, precipitated and translated *in vitro*. Samples of the cell-free products were reacted with anti-AMV gp85 serum<sup>19</sup>, immunoprecipitated and analysed on 15% SDS-polyacrylamide gels







**Fig. 2** *a*, Calibration of 5–20% sucrose gradient containing 50% formamide. Total RSV-infected chick embryo fibroblast mRNA (50  $\mu$ g) was denatured at 37 °C for 5 min in 50% dimethylformamide, 25% dimethylsulphoxide and fractionated on a 50% formamide sucrose gradient<sup>20</sup>. Centrifugation was for 12 h at 35,000 r.p.m. in a Beckman SW40 rotor at 20 °C. <sup>32</sup>P-labelled non-poly (A)-containing cytoplasmic RNAs (●) and <sup>3</sup>H-labelled RSV 30–40S RNA (▲) were mixed, denatured and fractionated on a parallel gradient. mRNA was recovered from the gradient fractions as described in Fig. 1*a* and translated in an L-cell system supplemented with 50  $\mu$ g crude rabbit reticulocyte initiation factors<sup>21</sup> per 50  $\mu$ l incubation. The <sup>35</sup>S-methionine incorporated into TCA-insoluble material in a 2- $\mu$ l sample of the cell-free system in response to RNA from the gradient fractions is, also indicated (■). *b* and *c*, SDS-polyacrylamide gel electrophoresis following immunoprecipitation with anti-AMV p12 serum and anti-AMV gp85 serum of the polypeptides synthesised in the L-cell system in response to RSV-infected chick cell mRNA fractionated on a 5–20% sucrose gradient containing 50% formamide. *b*, Samples (20  $\mu$ l) of the products made in response to selected fractions of gradient fractionated mRNA (*a*) were reacted with 5  $\mu$ l normal rabbit serum and incubated at 22 °C for 90 min. The rabbit IgG was removed by binding to protein A present on the surface of *Staphylococcus aureus*<sup>22</sup>, followed by centrifugation. This procedure was designed to reduce the background of nonspecific precipitation. The supernatant was reacted with 5  $\mu$ l rabbit anti-AMV p12 antiserum and the immunoprecipitates collected using the double antibody method<sup>6</sup>. An incubation containing no added RNA (*b*), and one containing virion 30–40S RNA (*M*) were treated in the same way. All the samples were run on the same 15% SDS-polyacrylamide gel and the gel was subjected to fluorography. Because of the differences in counts, track *M* was exposed for only 24 h whereas the remainder of the gel was exposed for 3 weeks. *c*, As for *b* except that immunoprecipitation was with anti-AMV gp85 serum and a different selection of fractions were translated.

mRNA constitutes only a small fraction of total mRNA activity, the viral proteins made *in vitro* were isolated by immunoprecipitation using various antisera raised against avian RNA tumour virus proteins.

### Fractionation of infected cell mRNA

Poly(A)-containing cytoplasmic RNA was isolated from c/o chick embryo fibroblasts transformed with non-defective Prague-C RNA, from uninfected chick cells, and from duck fibroblasts (which do not contain detectable endogenous viral sequences). These mRNAs were fractionated on 2–10% sucrose gradients containing 85% formamide. <sup>32</sup>P-labelled

18S and 28S rRNA and <sup>3</sup>H-labelled RSV virion 30–40S RNA were sedimented as markers in a parallel gradient. RNA from each of the gradient fractions was recovered and translated in the ascites cell-free system. The cell-free products were immunoprecipitated with antiserum raised against detergent-disrupted AMV, which contains antibodies against both the gs antigens and the envelope glycoproteins, and the immunoprecipitates were analysed by electrophoresis on SDS-polyacrylamide slab gels. RSV virion 30–40S RNA was translated, immunoprecipitated and electrophoresed together with the products made in response to fractionated mRNA.

Figure 1*a* shows that only one polypeptide is immunoprecipitated from cell-free incubations containing added virion

30–40S RNA, using either anti-AMV or anti-p12 sera. We have previously identified this as Pr76, the precursor to the viral structural proteins<sup>6</sup>. Because less radioactivity is present in the immunoprecipitates of material made in response to the fractionated mRNA, this region of the gel was autoradiographed for longer. This reveals that a background of proteins (notably actin) which is present in the material immunoprecipitated from all fractions, is also immunoprecipitated by control serum, and uninfected duck cell mRNA products. In addition to the background, there is a polypeptide which comigrates with marker Pr76 and is synthesised from an mRNA activity from RSV-infected cells which sediments slightly slower than the 28S RNA marker (Fig. 1a). On 85% formamide sucrose gradients, virion 30–40S RNA also sediments slower than 28S RNA.

A further polypeptide which we estimate to have an apparent molecular weight of 70,000 is immunoprecipitated by antiserum to disrupted virions from the products made in response to mRNA from infected cells. The mRNA coding for the 70,000 polypeptide sediments slower than the mRNA coding for putative Pr76. Virion RNA does not code for the 70,000 protein (Fig. 1a track M).

The 70,000 molecular weight polypeptide synthesised by RSV-infected cell mRNA is not immunoprecipitated by control rabbit serum, nor is it detected when uninfected chick or duck cell mRNA are translated and immunoprecipitated (data not shown). To characterise the 70,000 protein further we have used antisera raised against purified viral components. The 70,000-MW polypeptide was not immunoprecipitated by antiserum raised specifically against the group-specific antigen, P12, which precipitates Pr76 made *in vitro*<sup>6</sup>. Antiserum raised specifically against the major viral glycoprotein gp85, however, immunoprecipitated the 70,000 protein from the translation products of total unfractionated infected cell mRNA and of the 20–25S fraction. Figure 1b shows the immunoprecipitates obtained with anti-gp85 from the products made in response to 20–25S RNA from infected chick cells and uninfected duck cells. Although there is a high background it is clear that the 70,000 protein is only present in the product made by infected cell mRNA. The results of the immunoprecipitation experiments indicate that the 70,000 polypeptide may be a virus-specific molecule, and if so, that it may be related to the envelope glycoprotein gp85.

The experiment shown in Fig. 1 used 85% formamide sucrose gradients because they are reported to be highly denaturing<sup>17</sup> and we have previously found such conditions to be necessary to reduce mRNA–mRNA interactions to a minimum<sup>16</sup>. These gradients, however, do suffer the disadvantage that mRNAs larger than about 18S do not sediment at their true rate. Indeed, as already mentioned, <sup>3</sup>H-labelled 30–40S virion RNA sediments slower than 28S rRNA in these conditions. Thus, although the results presented in Fig. 1 do not allow us to assign accurate sedimentation constants to the different RNA fractions, they do indicate that the mRNA coding for putative Pr76 is different and separable from that coding for the 70,000-dalton protein.

To achieve a greater separation of the two mRNAs from RSV-infected cells, and a more accurate estimate of their size, we repeated the experiment but in this case used 50% formamide sucrose gradients and collected more fractions. To identify the products we translated RNA from selected fractions in an L-cell S30 and immunoprecipitated the products with the two specific antisera, against the purified gs antigen P12 and against purified viral glycoprotein gp85, mentioned above. To reduce the background of immunoprecipitated material which we found to be higher when using the monospecific antisera (Fig. 1b) we introduced a pre-immunoprecipitation step using control serum as described in the figure legend.

Figure 2a shows the position of 18S and 28S rRNA and 30–40S virion RNA sedimented on a 5–20% sucrose gradient containing 50% formamide, and also the <sup>35</sup>S-methionine incorporation into TCA-insoluble material when infected cell

mRNA was fractionated on a parallel gradient and translated in the L-cell system. Figure 2b shows that a polypeptide, which comigrates on SDS–polyacrylamide gel electrophoresis with marker Pr76, is immunoprecipitated by anti-P12 serum from the products made in response to an mRNA activity which cosediments with virion 30–40S RNA. Pr76 cannot be detected in the products made from mRNA sedimenting slower than about 28S.

When the translation products of infected cell mRNA in the L-cell system are subjected to immunoprecipitation with antiserum raised against AMV gp85, Pr76 is not detected in the immunoprecipitates (Fig. 2c). The 70,000-MW polypeptide can be seen, however, and is translated from an mRNA activity well separated from that coding for Pr76, sedimenting between about 20 and 28S. In spite of the pre-immunoprecipitation step, a considerable background still remains in the immunoprecipitates with anti-gp85 serum (Fig. 2c). Nevertheless immunoprecipitation of the 70,000 protein seems to be specific since when uninfected duck cell mRNA was translated and immunoprecipitated with the anti-gp85 serum, the background was still seen but the 70,000 protein was absent (for example see Fig. 1b); further, the 70,000 protein was not immunoprecipitated by the anti-P12 serum (Fig. 2b).

### A model for the translation of viral RNA

The data presented here suggest that in chick cells productively infected with non-defective RSV, the only RNA species which can actively direct the synthesis of the gs antigen precursor Pr76 is a polyadenylated RNA of the same size as virion 30–40S RNA. Whether the 30–40S cellular RNA species which codes *in vitro* for the synthesis of Pr76 is identical to virion 30–40S RNA, or whether there are two forms of viral RNA of this size, one of which is translated and the other is encapsidated, is unknown. Although we have not yet measured the amount of viral RNA present in the infected-cell mRNA, estimates based on previously published reports<sup>11,23–27</sup> indicate that there is no striking difference in the translational efficiency of cellular 30–40S RNA and virion 30–40S RNA in coding for Pr76. Some of the high molecular weight viral RNA from infected cells may originate from nucleocapsid where *in vivo* it is probably not available for translation. If all the 30–40S RNA were encapsidated, however, and there was a virus-specific mRNA smaller than 30–40S RNA directing the synthesis of Pr76 in these cells, we should have detected it.

In addition to the Pr76 viral polypeptide, a 70,000-molecular weight polypeptide has been detected in the translation products of mRNA from infected cells. The suggestion that this polypeptide is a viral-specific product and possible envelope glycoprotein precursor is based on the finding that it is immunoprecipitated by anti-disrupted virus serum (Fig. 1a) and by anti-gp85 serum (Fig. 2c), and not by control or anti-P12 serum (Fig. 2b). Further, it cannot be detected in the translation products of uninfected cell mRNA (Fig. 1b).

We are not aware of any example of a eukaryotic protein that, following synthesis *in vitro*, is extensively glycosylated. It is therefore not surprising that the protein made *in vitro* does not migrate with the reported mobility of viral gp85. Our results are consistent, however, with the observation that when RSV-infected cells are pulse labelled and cell lysates subjected to immunoprecipitation with anti-gp85 serum, an incompletely or non-glycosylated precursor to gp85 is detected (P70) and this has an apparent molecular weight on SDS–polyacrylamide gels of 70,000 (ref. 28). Since the antiserum used was from the same laboratory as that used in our study and the apparent molecular weights of the two proteins are similar it seems possible that our 70,000 protein is the non-glycosylated polypeptide moiety of gp85. It must be emphasised, however, that the identification of the 70,000-molecular weight polypeptide translated from infected cell mRNA is based solely on immunoprecipitation. Rigorous identification of the polypeptide must await the development of new methods which will enable us to purify sufficient virus-specific cytoplasmic mRNA to give



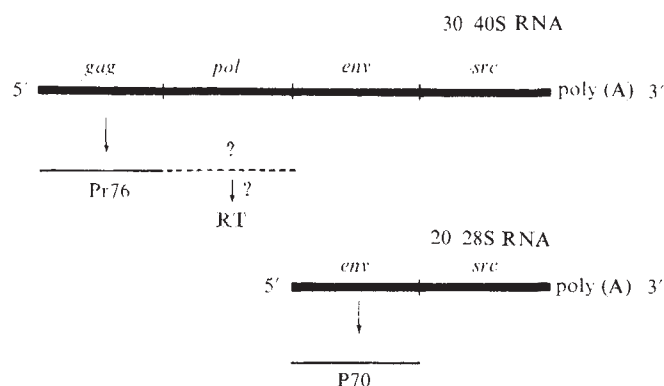


Fig. 3 Possible model for the synthesis of virion RNA proteins.

cell-free products which can be analysed by tryptic peptide fingerprinting.

The results presented here suggest that the two size classes of viral RNA that have been detected in the polysomes of infected cells by several groups both actively direct the synthesis of viral proteins. The finding that the mRNA activity sedimenting between 20-28S codes *in vitro* for a 70,000 polypeptide, but that virion RNA or virion-sized mRNA from infected cells do not code for this protein suggests that the two mRNAs have different coding activities. Possibly the 20-28S RNA represents the 3' half of virion 30-40S RNA and the corresponding internal initiation sites for protein synthesis present in virion RNA is inactive (see Fig. 3). Such a model is similar to those already presented for the synthesis of Semliki forest virus structural proteins<sup>29</sup>, polyoma virus capsid proteins<sup>16</sup>, and several plant virus capsid proteins. Bloemendal and colleagues<sup>31,35</sup> have reported similar experiments to those described here and have shown that different species of viral mRNA from cells infected with murine leukaemia virus (MuLV) have different coding activities. These experiments are also consistent with the model shown in Fig. 3.

How the *pol* gene is translated is still unclear. Arlinghaus *et al.*<sup>30</sup> have reported that a precursor to the viral proteins of molecular weight 200,000 is present in MuLV-infected cells and that this includes the structural proteins and possibly the reverse transcriptase. Kerr *et al.*<sup>31</sup> have synthesised a 180,000-molecular weight polypeptide *in vitro* in response to MuLV 30-40S virion RNA. This protein includes the tryptic peptides of the structural proteins, but no further components have yet been identified. It is likely that the high molecular weight protein found in the murine system is the product of read-through from the *gag* gene into the *pol* gene, but that this readthrough does not occur in every case. Such a model would be similar to that for the synthesis of bacteriophage Q $\beta$  coat protein and its readthrough product, the A<sub>1</sub> protein<sup>32</sup> and for the synthesis of Mengo virus proteins late in infection<sup>33</sup>.

While this manuscript was in preparation, Mueller-Lantzsch and Fan<sup>36</sup> reported that the only viral RNA species present in polysomes immunoprecipitated by anti-MuLV p30 serum from total infected cell polysomes has a sedimentation rate of 30-35S.

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**Note added in proof:** Since this manuscript was submitted other authors have reported that RSV subgenomic mRNAs from infected cells direct the synthesis of the viral glycoprotein when injected into chick cells<sup>37</sup> and oocytes<sup>38</sup>.

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## letters to nature

### Cyg X-3: $\gamma$ rays from a magnetic accretion disk

THE recent observation<sup>1</sup> of  $\sim 100$ -MeV  $\gamma$  rays from Cygnus X-3, which have a comparable luminosity to the X-ray emission and which vary in phase with the 4.8-h period displayed by the X-ray and infrared emission, has been taken<sup>2, 3</sup> as support for the suggestion<sup>4</sup> that the X-ray emission is produced by a very young pulsar in a short period binary system. In this letter, we show that the observed  $\gamma$ -ray luminosity can be produced by an accretion disk around a non-magnetic neutron star or

low-mass black hole, in a magnetised corona which accelerates relativistic electrons that decay by inverse Compton losses modified through the photon-photon and pair-annihilation processes.

The dominant dissipative mechanism in the disk is assumed to be due to magnetic fields. The shearing motion of the disk increases the azimuthal field strength in the disk until the magnetic pressure is comparable with thermal (or radiation) pressures inside the disk. We then expect the field lines to bow apart from the disk to form a magnetic corona above the disk as described by Parker<sup>5</sup>. This process may also turn sufficient

of the azimuthal field into poloidal directions so that a dynamo mechanism operates. Since the Alfvén speed in the corona exceeds that in the disk (where most of the matter resides) and since the lengthscales associated with field reversals should be comparable in both regions, we expect most of the dissipation of magnetic energy (through field annihilation and particle acceleration) to take place in the corona and not in the disk itself. There is a self-consistent equilibrium situation in which the disk is kept at X-ray temperatures producing photons with energy  $E_x \sim 10$  keV. The relativistic electrons (energy  $= \gamma m_e c^2$ ) generated by the field annihilation, lose energy predominantly by inverse Compton losses by up-scattering the X-ray photons to  $\gamma$ -ray energies. Taking an X-ray luminosity<sup>6</sup> of  $L_x \sim 5 \times 10^{37}$  erg s<sup>-1</sup> to be coming from the inner part of a disk, a region of radius  $R \sim 3 \times 10^6$  cm, we find that the energy density of X-ray photons is  $\epsilon_x \sim 3 \times 10^{13}$  erg cm<sup>-3</sup> with corresponding X-ray photon number density  $n_x \sim 2 \times 10^{21}$  cm<sup>-3</sup>. For these parameters, and for  $\gamma_2 = 10^{-2}$   $\gamma \leq 1$ , the energy loss timescale for an electron to inverse Compton scattering is  $t_{ic} \sim 10^{-8} \gamma_2^{-1}$  and the corresponding radiation path length is  $\lambda_{ic} \sim ct_{ic} \sim 3 \times 10^2 \gamma_2^{-1} \ll R$  (for standard disk densities<sup>7</sup>, energy loss in the rough nuclear collisions is negligible). For synchrotron losses to be unimportant the magnetic field strength must satisfy  $B^2/8\pi \leq \epsilon_x$ , that is  $B \leq 3 \times 10^7$  G. The equipartition field in a standard accretion disk<sup>7</sup> at the above radius is  $\leq 4 \times 10^7$  G. We may expect the magnetic field to be somewhat less in the corona and, hence, take  $B = 10^7$  G.

To produce the required  $\gamma$ -ray photons with energy  $E_\gamma \sim 100$  MeV we require  $\gamma^2 \sim E_\gamma/E_x$ , or  $\gamma_2 \sim 1$ . When  $\gamma > m_e c^2/E_x \sim 50$ , the Klein-Nishina cross section is relevant and the energy of the  $\gamma$  ray produced in a collision is  $E_\gamma \sim \gamma m_e c^2$ . The energy loss timescale for an electron with  $\gamma_2 \gg 1$  is  $t_{ic}^* \sim t_{ic} \gamma_2^2$ .

Assuming that magnetic field reconnection takes place at a velocity  $\sim 0.1 V_A$ , where  $V_A$  is the Alfvén velocity in the corona<sup>8</sup>, we may estimate the induced electric field to be  $E \sim (0.1 V_A/C)B$ . The particle density in the corona is sufficiently low that we may take  $V_A \sim c$  and estimate  $E \sim 10^6$  units. If the particle acceleration occurs over a length,  $\lambda$  cm, we have that  $\gamma_2 \sim 10^{-3} eE\lambda/(m_e c^2) \sim 5\lambda$  (ref. 9). For self-consistency we demand that  $\lambda \leq \lambda_{ic}$  and hence that  $\gamma_2 \leq 0$ . It seems possible, therefore, that the initial photons produced have energies  $\geq 100$  MeV. We note, however, that photons with energies  $E_\gamma \gtrsim E_s \sim 4(m_e c^2)^2/E_x \sim 100$  MeV can undergo photon-photon interactions<sup>10</sup>, with cross section  $\sim \sigma_T$ , the Thomson cross section, to produce electron-positron pairs with  $\gamma_2 \geq 1$ . The optical depth of such  $\gamma$  rays in our region is  $\tau_\gamma \sim n_x \sigma_T R \sim 10^3$ . The pairs so produced then lose their energy by inverse Compton interactions (on a timescale  $\sim t_{ic}^* \sim \gamma_2/(n_x \sigma_T c)$ ) at a rate faster than they annihilate (on a timescale  $\sim 10^2 \gamma_2/n_x \sigma_T c$  where  $n_\pm$  is the electron-positron number density<sup>11</sup>). (We shall show that  $n_\pm \sim n_\gamma$  and since  $L_x \sim L_\gamma$  then  $n_x \sim 10^4 n_\gamma$ ). In this way, all photons are degraded to energies  $\sim E_s$  before escaping from the corona. This also implies that the rate of formation of electron-positron pairs is comparable to the rate of production of  $\sim 100$ -MeV photons. The timescale for escape of photons is  $t_p \sim R/c \sim 10^{-4}$  s whereas the annihilation timescale (now with  $\gamma \sim 1$ ) is  $t_\pm \sim 1/(n_\pm \sigma_T c)$ . If  $n_\gamma$  is the number density of  $\gamma$ -ray photons with  $E_\gamma \sim 100$  MeV, therefore, in a steady state  $n_\pm/n_\gamma \sim t_\pm/t_p$  and hence  $n_\pm^2 \sim n_\gamma/(R\sigma_T)$ . Assuming a  $\gamma$ -ray luminosity  $L_\gamma \sim 10^{37}$  erg s<sup>-1</sup> we find that  $n_\gamma \sim 10^{17}$  cm<sup>-3</sup> and hence that  $n_\pm \sim 2 \times 10^{17}$  cm<sup>-3</sup>. The annihilation of the electron-positron pairs gives rise to a line in the region of 500 keV which contains a photon flux comparable with that in  $\gamma$  rays, that is a luminosity  $L_\pm \sim (m_e c^2/E_\gamma)L_\gamma \sim 10^{35}$  erg s<sup>-1</sup>.

Once the  $\gamma$  rays have been sufficiently degraded they can escape from the coronal region. About a half of them can escape to infinity but the rest enter the accretion disk itself. From the standard disk model we find the surface density of the disk to be  $\sim 5$  g cm<sup>-2</sup>, so that the  $\gamma$  rays are absorbed by the disk. They heat the disk to X-ray temperatures (thus en-

suring the self-consistency of our assumption). In this model we find, therefore, that the rough equality of  $L_x$  and  $L_s$  arises naturally.

We can further examine why this process produces  $\gamma$  rays for a disk model of Cyg X-3, but may not for other sources in which disks may be present. From the standard disk model, the magnetic field strength in the disk in the neighbourhood of the central regions is  $\sim 10^8 (M/M_\odot)^{-1} (R/3R_s)^{-1}$  G (where  $M$  is the mass of the central object and  $R_s = 2GM/c^2$ ) independent of the accretion rate. If, therefore,  $\epsilon_x$  falls much below the value we find that for Cyg X-3 the dominant loss process for the relativistic particles is synchrotron radiation which produces photons in the X-ray range. Thus, if the luminosity of the X-ray source is too low, or if the area of the emitting region is too high (for example, for a  $10M_\odot$  black hole in Cyg X-1), the energy is radiated almost entirely as X rays, some thermally from the disk and some non-thermally as synchrotron radiation from the corona.

An irregular time variability, or the occasional disappearance of the  $\gamma$ -ray emission of Cyg X-3, could occur if  $\dot{M}$  (and consequently  $\epsilon_x$ ) drops below the value required for inverse Compton losses to dominate over synchrotron, or if  $\dot{M}$  increases to a value where the total luminosity approaches the Eddington value and disk symmetry is destroyed. In both cases, one could expect the  $\gamma$ -ray emission to disappear while the X-ray emission remains, albeit with somewhat different spectral characteristics.

The 4.8-h variation in the observed  $\gamma$ -ray flux can be produced in the same manner as the X-ray variation, using the model of Pringle<sup>12</sup>. In this model, the X-ray variation is produced by the variation with binary phase of the electron scattering optical depth to the X-ray source through a dense wind emanating from the companion star. We expect the  $\gamma$ -ray variation to be more pronounced, since the X-ray variability is smoothed somewhat by the backscattering of X-ray photons. On the other hand, backscattered  $\gamma$ -ray photons are degraded by Compton recoil.

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## Are striations on Phobos evidence for tidal stress?

VIKING orbiter photographs of Phobos, the inner satellite of Mars, reveal a curious series of striations or grooves over much of the surface<sup>1</sup>. These features cross over large eroded craters but are in turn interrupted by very small well defined craters, which indicate an origin substantially later than the time of formation of the satellite itself when the large scale cratering presumably occurred. The grooves have no obvious connection with the 'crater chains' also seen in Phobos, and their nearly parallel spacing seems to rule out an origin due to primary or secondary impacts. We suggest that some of these features are related to readjustment of the satellite's figure with increasing tidal stress as the orbit evolves inwards under the action of tidal friction.



The present rate of orbital decay<sup>2</sup> suggests that Phobos's original orbit had a radius  $a \sim 5R$  ( $R$  is martian radius) and that the present orbit, at  $2.76R$ , will decay to the martian surface (unless the satellite breaks up first) within  $\sim 10^8$  yr. The tidal (plus centrifugal) stress,  $\sigma \propto a^{-3}$ , has already increased by a factor of  $\sim 6$  since the origin of Phobos.

The magnitude of  $\sigma$  is now of order  $\Omega^2 \rho r^2 \approx 10^5$  dyn cm<sup>-2</sup>, where  $\Omega$  is the angular velocity,  $\rho$  the density, and  $r$  the radius of the satellite. If Phobos has a deep regolith layer of material similar to lunar dust, one might expect a material strength<sup>3</sup> near the surface as low as  $10^3$ – $10^4$  dyn cm<sup>-2</sup>. Thus even if the interior were perfectly rigid, tensile stress fields in the weakly cohesive surface regolith might give rise to patterns of parallel cracks. With the addition of a mechanism for the partial readjustment in the figure of Phobos under tidal stress, the surface cracks could then be widened into the observed series of grooves or graben.

For example, slow viscous creep due to subsurface permafrost could allow tensile cracks to widen. Spacecraft perturbations indicate a density<sup>4</sup> for Phobos of about 2 g cm<sup>-3</sup>. This low value, together with the satellite's observed photometric and polarimetric properties<sup>5</sup>, suggests a carbonaceous chondritic and hence water-rich composition, so that permafrost is not unreasonable. Shock waves due to impacting bodies might also enhance the readjustment of the figure.

If Phobos is homogeneous, its low mean density places it inside the classical Roche limit for the gravitational instability of a fluid body (ref. 6). Therefore, either the satellite has a denser core (we thank Dr S. Dermott for clarifying this point) or, if homogeneous, it possesses sufficient rigidity to prevent full readjustment on the timescale of orbital evolution ( $\sim 10^8$  yr).

One very prominent series of grooves is arranged nearly concentrically around the tidal equator (normal to the satellite's long axis), which orientation is to be expected from tidal stress theory<sup>7</sup>. Shear failure at moderate depths, where the principal stresses are all compressive, might give rise in addition to transcurrent faults, and there is perhaps an indication of this in some of the Phobos pictures showing sets of obliquely intersecting striations.

If the width of the grooves is in fact due to tidal readjustments of the figure of Phobos, then the older craters should be systematically deformed from their initial nearly circular shapes. Craters near the tidal equator would be elongated parallel to the satellite's long axis. A similar suggestion was made by Öpik<sup>8</sup> with respect to the Moon, but the effect for Phobos would be more pronounced.

Striations and other deformations due to tidal stress should be nearly absent on Deimos. The outer satellite has always been beyond the synchronous distance ( $6.0R$ ) and at present ( $a=6.92R$ ) has a tidal stress about 60 times weaker than that for Phobos.

An investigation is being made of the possible role of subsurface ice in producing the detailed morphology and distribution of tidal stress features on Phobos.

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## Geomagnetic storms and electric fields in the equatorial ionosphere

LARGE variations of the geomagnetic field observed at ground level during a geomagnetic storm are generally attributed to various magnetospheric changes following the arrival of the solar plasma ejected during the solar flare. Using the direct measurements of equatorial electric field during a geomagnetic storm, it is shown here that the large decrease in the field observed near the dip equator is due to the reversal of the equatorial electrojet current. This event is caused by the imposition of an additional westward electric field on the equatorial ionosphere which was originated by the interaction of solar wind with the interplanetary magnetic field.

A standard type of geomagnetic storm consists of a sudden increase of the horizontal ( $H$ ) field at ground level within a few minutes at all world stations (called storm sudden commencement—SSC) followed by the initial phase (IP) for a few hours when the  $H$  field is above normal. The main phase (MP) of the storm is supposed to start when the  $H$  field decreases below the pre-SSC level and ends when  $H$  reaches the minimum value. The recovery phase follows the main phase and may last one or two days. The history of a particular storm can be identified better by the temporal changes of the equatorial  $D_{st}(H)$  values rather than from temporal variation of  $H$  at a particular station.

The first explanation of the various effects of the geomagnetic storm was by Chapman and Ferraro with the concept of neutral ionisation moving from the Sun through the geomagnetic cavity. The SSC was explained in terms of an enhancement of the electric current induced on the surface of the cavity by the ion stream, and the MP was caused by a ring current surrounding the Earth. Dessler<sup>2</sup> suggested the SSC to be produced by hydromagnetic shock wave generated at the frontal surface. Rastogi and Trivedi<sup>3</sup> showed that the amplitude of SSC( $H$ ) at low latitudes is greatly enhanced in a way similar to the enhancement of  $\Delta H$  within the equatorial electrojet region, and thus the SSC near the dip equator was suggested to have a significant contribution due to ionospheric currents. Akasofu and Chapman<sup>4</sup> have shown that polar geomagnetic storm can greatly enhance the equatorial electrojet current. Rastogi<sup>5</sup> has shown that the changes of  $H$  field during SSC at a low-latitude station are due to the imposition of an additional electric field over the equatorial ionosphere causing additional SSC-associated electrojet currents. Onwumechilli *et al.*<sup>6</sup> have shown that during moderately disturbed periods, the polar  $S_q$  variation ( $S_q^p$ ) is enhanced and the electric field associated with it affects the equatorial electrojet by suppressing it. Further, the substorm-associated electric field driving the eastward current in the auroral zone causes an enhancement of the equatorial electrojet. An experimental verification of the coupling between equatorial and auroral electric fields has been recently reported by Kelley *et al.*<sup>7</sup>, when the eastward electric field measured at the equatorial station Jicamarca by the Doppler shift of VHF scatter is very well correlated with the westward electric field at auroral station College Alaska measured by balloon-borne detectors.

Woodman<sup>8</sup> has recently described the vertical as well as eastward F-region drifts at Jicamarca. At these regions of the ionosphere, these drift velocities are a direct measurement of the electric field in a direction orthogonal to drift and to the direction of the magnetic field. Thus a technique of continuous monitoring of ionospheric electric fields over

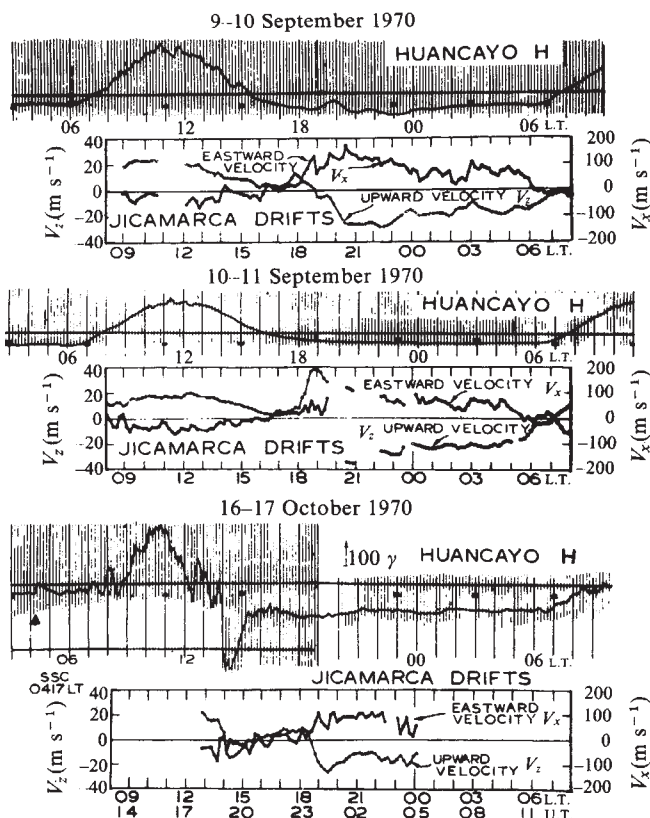


Fig. 1 Comparison of the F region upward and eastward drift velocities at Jicamarca with the magnetograms at Huancayo.

the dip equator has been provided through VHF scatter radar. The data were presented for three days 9–10 September 1970, 10–11 September 1970 and 16–17 October 1970. The first two days, 9–10 and 10–11 September 1970 were geomagnetically very quiet while 16–17 October 1970 was one of the five disturbed days of the month. A sudden commencement type of geomagnetic storm had occurred on 16 October 1970 at 0917 UT (0417 LT). In Fig. 1, these data of upward velocity ( $V_z$ ) and eastward velocity ( $V_x$ ) are compared with the corresponding magnetogram of Huancayo.

In Fig. 1, the H field at Huancayo varies very smoothly on 9–10 and 10–11 September 1970. In the evening of 10 September 1970, the vertical drift (or eastward electric field) had a very prominent peak around 1900 LT before the usual evening reversal of its direction. No effect of this sudden increase of the electric field could be seen in the magnetogram because the E region plasma had greatly decreased by 1900 LT and so no significant currents could be produced due to the increase of the electric field. On 9–10 September 1970, the evening peak of the vertical drift was absent. The magnetogram for 16–17 October 1970 shows a sudden increase of the H field at 0417 LT due to sudden start of geomagnetic storm. There is a remarkably large and sudden decrease of the H field between 1400 LT and 1530 LT. The vertical drift at Jicamarca shows a large excursion such that the drift was downward between 1400 LT and 1540 LT. Thus, this large decrease of H field near the dip equator during the geomagnetic storm period seems to be due to electric currents in the electrojet region itself due to imposition of a reversed electric field. As described by Woodman<sup>8</sup>, the eastward drift shows consistent behaviour from day to day even during a magnetic disturbed day. The variations of the geomagnetic field are further studied in detail in Fig. 2 which depicts the varia-

tions of the H field at Huancayo, Fuquene and Huancayo minus Fuquene in American zone and at Kodaikanal, Alibag and Kodaikanal minus Alibag in Indian zone. The corresponding variations of the  $D_{st}(H)$  values, the auroral electrojet (AE) index, interplanetary magnetic field magnitude ( $B$ ) and the latitude ( $\theta$ ) are also shown. The latitude  $\theta = -90^\circ$  shows the IMF vector entirely southward while  $\theta = +90^\circ$  shows IMF vector entirely northward.

It is seen that the  $D_{st}$  became negative at 1130 UT which signifies the starting of the main phase of the storm. This tallies very well with the variations of H at Kodaikanal and at Alibag. The difference fields Kodaikanal minus Alibag is very low during the entire period suggesting that most of the storm effects at Kodaikanal were of magnetospheric origin, and this is consistent with the fact that the storm period was local night for these stations. The onset of MPO does not seem to have affected the Huancayo H values which shows a peak before noon. The disturbance field was thus too small to cancel the equatorial electrojet field at Huancayo. At 1400 LT (1900 UT) the H field at Huancayo shows a sharp decrease but no change was noticeable at Fuquene. Thus, this  $\Delta H$  at Huancayo seems to be entirely due to change in the equatorial electrojet current and not due to any changes in the magnetospheric currents. It is remarkable that the Huancayo

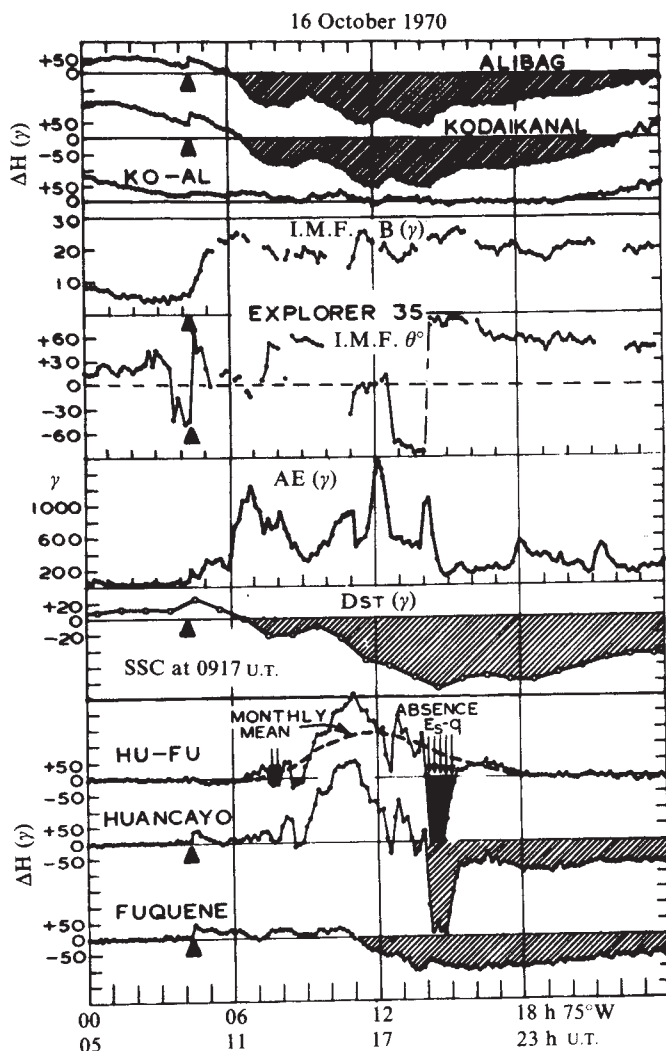


Fig. 2 Comparison of the magnetic field variations at low latitudes during a geomagnetic storm with the auroral index and the interplanetary magnetic field parameters.



ionograms showed absence of the  $q$  type of  $E_s$  layer confirming that the horizontal electric field at the E region over Huancayo between 1400 and 1530 LT was reversed to westward. Examining the AE index, one notices increase of the index around 0700 LT, 1200 LT and 1400 LT. The intense activity of auroral electrojet at 0700 LT did produce some decrease of the H at Huancayo between 0800 and 0900 LT. The large increase of AE around 1200 LT decreased the H field around 1230 LT. The most prominent decrease of H field at 1400 LT is associated with the sudden increase of the auroral index at 1400 LT.

Examining the changes of IMF parameters one notices that after the sudden start the magnitude ( $B$ ) had increased from its about usual value of  $5 \gamma$  to above  $20 \gamma$  and remained high for the rest of the day. This high value of IMF made the interaction of the field with the solar wind most effective. The direction of the field perpendicular to the ecliptic ( $\theta$ ) shows sudden change at SC and later at 1200 LT. The most remarkable change occurred at 1400 LT when the field flipped from almost the southward to the northward direction. The field remained northward for the rest of the day. Thus the component of the IMF perpendicular to the ecliptic,  $B_z$ , component interacting with the solar wind plasma moving practically along the Sun-Earth direction produces a  $V \times B_z$  field along the east-west direction. A sudden reversal from southward to northward direction would present a strong westward electric field on the dayside of the Earth. The large increase of the AE index and the reversal of the equatorial electrojet at 1400 LT are ascribed to the sudden flipping of the  $B_z$  component of the interplanetary magnetic field.

Similar effects of the sudden reversals of the  $B_z$  component on the equatorial ionosphere have been previously described by Rastogi and Patel<sup>9</sup>. Similar effects seen during geomagnetic storms show, for the first time, that the observed magnetic field changes near the magnetic equator during geomagnetic storms have significant contribution due to the changes of the electrojet currents in the ionosphere through the modulation of the east-west electric field in the ionosphere. These changes at the equator are suggested to be of the solar wind origin manifested through the changes of the auroral electrojet currents.

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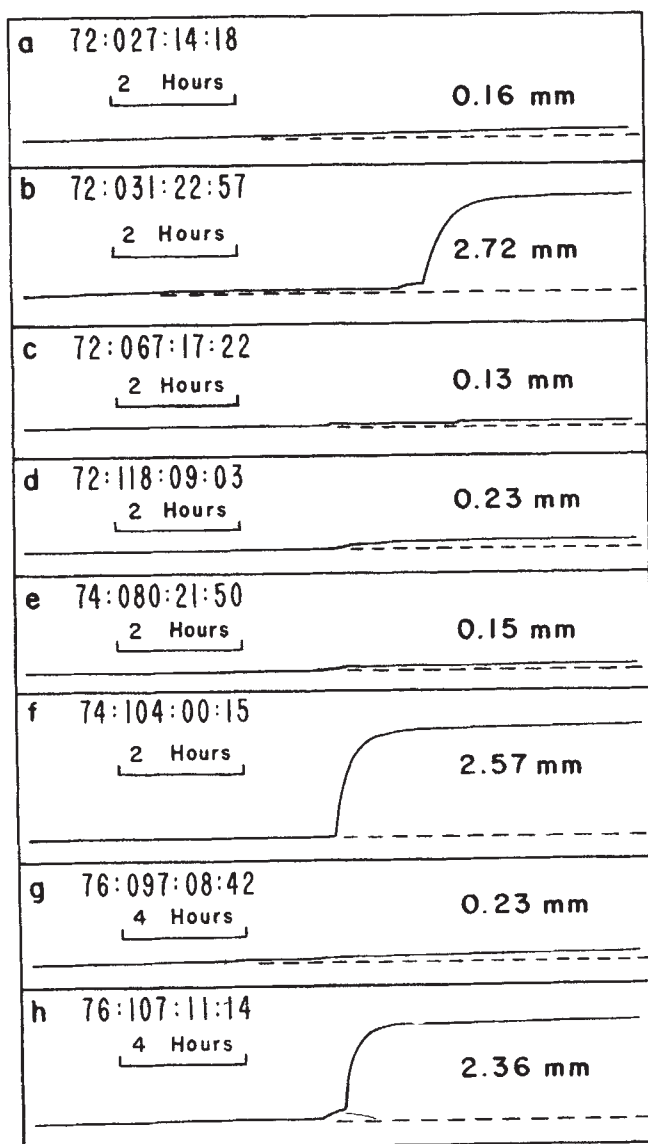
## Bimodal distribution of creep event amplitudes on the San Andreas fault, California

EPISODIC fault creep, at several instrument sites along the San Andreas and associated faults in central California consists of a few small and large slip events per year generally superimposed on a background of gradual yielding at low rates<sup>1-3</sup>. Most of the events are aseismic, but a few minor displacement steps have occurred in association with local earthquakes<sup>12</sup>. After removal of earthquake steps, event lists for several sites include significant numbers of small events about an order or magnitude below the typical 1-4-mm amplitude range for large events<sup>1, 3</sup>. Recent experimental rock-deformation

results demonstrate that under biaxial loading some rocks show episodic slip on pre-cut surfaces<sup>9, 10</sup>. It is not yet clear how the laboratory and field observations are related, but the data presented here indicate that episodic fault creep in nature may be more complex than previously realised. In light of the laboratory results, it is more important than ever to consider all the details of the field data concerning fault creep.

The performance of various types of non-wire creepmeters is usually adequate for monitoring large creep events, but these higher friction instruments generally produce incomplete or unreliable results at the level of sensitivity necessary for study of small events<sup>2, 12</sup>. Several invar wire creepmeters with low friction and displacement resolution of 0.05 mm or better have, however, been operated nearly continuously within the network since 1972 (ref. 1). At sites characterised by episodic

**Fig. 1** Tracings from on-site strip-chart records produced at XMR1 creepmeter (36°35.6'N, 121°11.2'W). Onset times for each event are given by yr, Julian d, h and min (GMT). Trace *f* shows a typical large creep event with 80% of the slip occurring in about 1 h. Traces *b* and *h* show similar events except that both are preceded by small precursory movements of about 0.2-0.3 mm. Trace *d* shows an isolated small event with a rise time similar to that for most large events. Although trace *d* represents the majority of small events, a few with shorter rise times (traces *c* and *e*) and with rise times of several hours (traces *a* and *g*) also occur.



creep, the low-friction wire creepmeters confirm the general pattern of large isolated creep events recorded on the other instruments, but show additional interesting detail concerning the nature of small events.

Records combined from one wire (XMR1)<sup>1,3</sup> and four rod-type creepmeters (MRC, MRB, MRR, MRW)<sup>2,3</sup> clustered along the San Andreas fault trace on Melendy Ranch (36°35'N, 121°11'W) show that most of the large events have rise times of the order of 1 h. These events often propagate at several hundred metres per hour along the entire 1.2-km fault segment covered by the five-station network<sup>1-5,7</sup>. From these and similar observations elsewhere it is clear that large creep events typically involve fault lengths on the order of a kilometre in episodic aseismic slip of a few millimetres amplitude<sup>6,8</sup>. Whatever the actual depth of creep events, it is also clear from consideration of source length alone that considerable volumes of material are involved in the process.

One of the most complete samples of creep event activity is contained in the 1972-76 records from the XMR1 wire creepmeter<sup>1,3</sup>; several examples are shown in Fig. 1. The small events usually have rise times similar to those for large events, but a few small events with shorter rise times (Fig. 1c, e) and with rise times of several hours (Fig. 1a, g) are also recorded. The distribution of event amplitudes at XMR1 is distinctly bimodal (Fig. 2). Another interesting feature is that small events at XMR1 preferentially occur during periods of several days just before large events (Fig. 3).

The surprising gap in the distribution of creep event sizes indicates that small events are not necessarily low-amplitude versions of the identical process acting over the same area of fault surface that produces large events. This being the case, there are at least two possible alternatives. The creepmeters may be measuring either the results of the same process operating on two distinctly different scales or the time-displacement signatures of distinctly different failure or propagation processes.

Strong evidence supports the first alternative in that large events usually propagate with a certain degree of regularity across several instrument sites<sup>5,7</sup>, whereas small events usually appear at only one creepmeter at a time. Consequently, for small events that are observed, the point at which rupture begins must be very close to the instrument, and the minor slip probably is confined to a relatively small area of the fault surface. The lack of clear evidence for propagation of some of the smallest events over greater distances ( $\geq 0.5$  km), however, could be because their amplitudes are commonly below the resolution of the non-wire creepmeters at sites adjacent to XMR1.

The concentration of small events just before large events indicates that the former are preferentially triggered at stress levels only slightly lower than those necessary for triggering large events. Small events recorded at XMR1 occasionally blend immediately into large events (Fig. 2b, h), perhaps owing to triggering of slip on a larger fault surface by the initial yielding of a small source area. The time relation reinforces the idea that small and large events result from the same process, with amplitudes determined by the area of the slip surface.

In contrast with this interpretation for the data from central California, results from a strainmeter array in Iran indicate that a small creep event (slip amplitude of about 10  $\mu$ m) propagated more than 0.8 km along the fault break of the 1968 Dasht-e-Bayaz earthquake, as reported by King and others<sup>11</sup>. The strain results from Iran plus the wider range of rise times for small events than for large events at XMR1 provide some support for the possibility that different failure or propagation processes might be involved.

The explanation for large and small creep events involving a bimodal distribution of source areas implies a heterogeneous distribution of static friction along the fault surfaces that fail in episodic aseismic slip. Large events are perhaps prevented until conditions are favourable for nearly uniform propagation

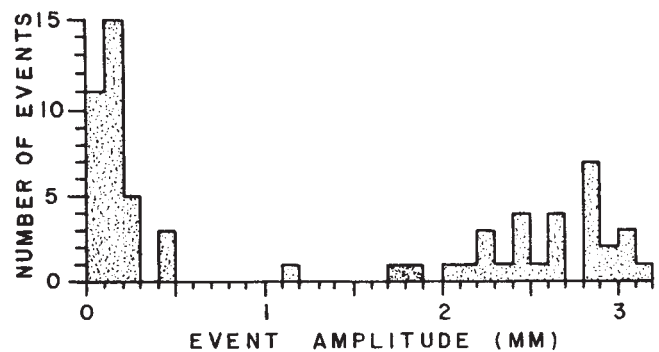
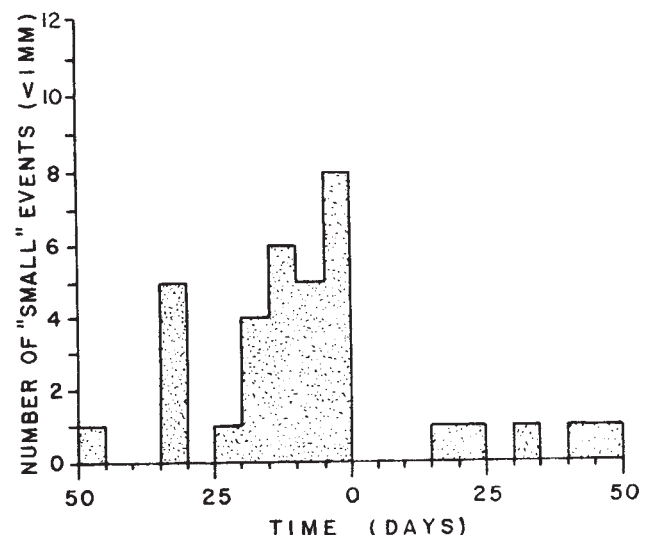


Fig. 2 Histogram of the number of events at wire creepmeter site XMR1 through 16 April 1976, as a function of slip amplitude. Similar bimodal distributions of event amplitudes have been demonstrated for several other sites but are less well documented on non-wire creepmeter records. A decrease in the number of events within the amplitude range 0-0.1 mm compared with the next higher range is probably a reflection of the limits of resolution; a number of small events with amplitudes less than 0.05 mm undoubtedly escape detection.

of aseismic slip over the entire large-event source area. The final stages of progress toward this condition probably are marked by a nearly uniform stress level slightly below that necessary for generating large events, but sufficient for initiating minor creep events across small, isolated, relatively weak patches of the fault surface within the source area for the impending large event. The small source areas are viewed as being quasi-permanent in position and dimensions. Slip action would cease after each small event until the general stress reached the threshold value for production of large events. Although many minor-event sources could be widely scattered across the large event area, only those adjacent to or encompassing a creepmeter site would produce detectable offsets. Activity on more distant small-source patches could produce certain small events with extremely long rise times.

The expected behaviour of the proposed bimodal source-area system would seem to fit the observed distribution of event amplitudes, the concentration of small events during short periods just before large events, and perhaps the wide range of small-event rise times. The final small event leading to

Fig. 3 Distribution of onset times for small creep events in five-day increments before and after large creep events, from XMR1 records. The zero point on the time scale represents onset times for all large events recorded at the site.





general failure over the large-event source area could occasionally occur near a creepmeter, producing a composite event on the record for that site.

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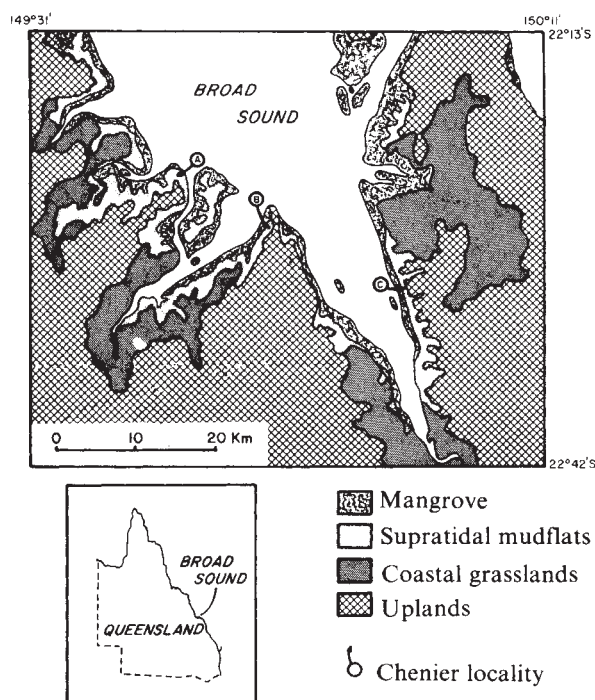


Fig. 1 Map of the Broad Sound area showing chenier locations from which oysters were obtained for this study.

## Loss of boron from shells during weathering and possible implications for the determination of palaeosalinity

THE abundance of boron in sediments is frequently taken as an indication of palaeosalinity<sup>1–5</sup>. Because boron is readily absorbed by clay minerals, determinations are generally undertaken on argillaceous sediments, particularly those containing abundant illite, though some analyses are carried out on calcareous sediments. One of the basic assumptions of the boron technique for the determination of palaeosalinity is that there is little or no mobility of boron after deposition, as any mobility might lead to enrichment or depletion of the original boron, and an unreliable value for the palaeosalinity.

A recent study of a Holocene shell sequence from the central Queensland coast indicates a systematic decrease in boron content of the shells with increasing age. This could conceivably be due to a progressive change (that is, decreasing salinity) of the original depositional environment, but other lines of evidence do not support this. It seems more likely that the loss of boron is a consequence of sub-aerial weathering. This suggests that erroneous boron palaeosalinity results may be obtained from calcareous sequences which have been subjected to sub-aerial weathering.

During a study of the sedimentology<sup>7</sup> and geochemistry<sup>8</sup> of sediments at Broad Sound, on the central Queensland coast (Fig. 1), a series of elongate ridges (cheniers) were found, composed predominantly of whole and fragmentary shells. Radiocarbon dating of the shell carbonate revealed that the ridges became progressively older landward, ranging in age from  $1,020 \pm 70$  yr BP to  $5,450 \pm 85$  yr BP (ref. 8). This sequence offered a possible opportunity for quantifying the rate at which geochemical changes take place in calcareous material under conditions of sub-aerial tropical weathering, or alternatively, for establishing the extent of environmental (temperature, salinity) changes which have taken place in the Broad Sound estuarine system during the last 5,000–6,000 yr. Because the chemistry of shells varies markedly with different phyla and species, the study was restricted to oyster shells, a bulk sample for analysis being prepared from 30–40 small oyster shells of approximately

the same diameter, collected from eleven of the cheniers. In addition, modern oyster shells were also collected for analysis from an adjacent intertidal site. All shells were cleaned for 30 minutes in distilled water in an ultrasonic bath before analysis. The results of the boron analyses together with the radiocarbon ages and the proportion of acid-insoluble material are given in Table 1. X-ray diffraction analysis, carried out on all samples showed that the shells are composed of low-magnesian calcite with no significant change in the position of the main calcite peak. But the boron content varies somewhat; it is evident from a plot against radiocarbon age (Fig. 2) that boron decreases with age. Over a period of about 5,000 yr the mean boron content of the shells decreases to 40% of the present day content. Determination of the correlation coefficient indicates that the negative correlation between boron and age has a 99% confidence level.

Table 1 Age and composition of oyster shells collected from cheniers, Broad Sound, Queensland

| B.M.R.<br>No. 71636 | Age<br>(yr BP) | Boron<br>(p.p.m.) | %<br>acid insolubles |
|---------------------|----------------|-------------------|----------------------|
| 205                 | 0              | 17                | 0.08                 |
| 100                 | 1170           | 15                | 0.28                 |
| 141                 | 1640           | 16                | 0.04                 |
| 103                 | 2100           | 16                | 0.87                 |
| 138                 | 2140           | 15                | 0.26                 |
| 202                 | 2480           | 15                | 0.13                 |
| 039                 | 2910           | 17                | 0.53                 |
| 139                 | 2930           | 14                | nd                   |
| 106                 | 2960           | 13                | 0.08                 |
| 203                 | 3465           | 12                | 0.46                 |
| 107                 | 4060           | 14                | 0.15                 |
| 204                 | 4520           | 12                | nd                   |

nd, Not determined (insufficient sample available).

Radiocarbon ages determined by H. A. Polach, Radiocarbon Laboratory, Australian National University, and R. Gillespie, Radiocarbon Laboratory, Sydney University. Boron was determined spectrophotometrically (using the organic reagent curcumin) by the Analytical Chemistry Section of the Australian Mineral Development Laboratories. Ten replicate analyses indicate a precision of  $\pm 1$  p.p.m.

Per cent acid insoluble determined by E. Kiss, Research School of Earth Sciences, Australian National University.

This change in concentration might conceivably be attributable to a progressive increase in salinity at Broad Sound over the past 5,000 yr. Kershaw<sup>9-11</sup> has suggested from palynological evidence that from about 6,000 to 3,000 yr BP the climate of North Queensland was significantly warmer and probably also wetter than the present-day climate. Broad Sound is situated at the confluence of four large estuaries but at the present day there is sporadic freshwater inflow only during the summer months (December to February) and even during this period, measurable dilution in the vicinity of the sampling sites lasts for only a few days. For almost the whole of the year, the water in Broad Sound and its associated estuaries shows complete vertical mixing and has a salinity of about 36 parts per thousand. The increase in rainfall required to have significantly affected the salinity of the Sound would therefore have needed to be of a far greater magnitude than that indicated by Kershaw's work. In addition, comprehensive stratigraphic studies<sup>7</sup>, coupled with radiocarbon dating<sup>12</sup>, indicate that since sea level stabilised at about 5,000-6,000 yr BP, depositional conditions have remained essentially constant. Therefore, it seems unlikely that the change in boron content can be attributed solely to a progressive increase in salinity at Broad Sound from about 5,000 yr BP to the present.

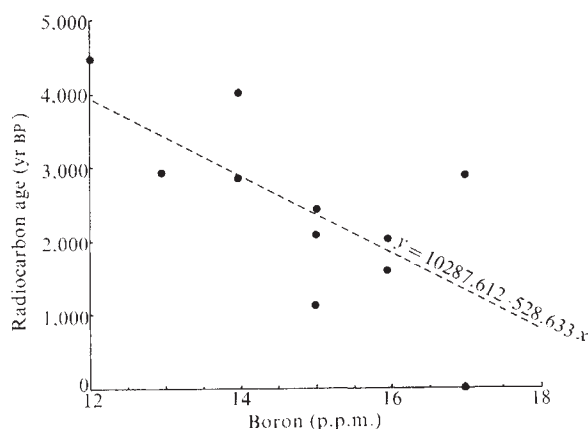


Fig. 2 Plot showing the progressive decrease in the boron content of oyster shells with increasing age.

An alternative explanation is that the decrease in boron content has occurred as a result of weathering. Because of the low concentration of boron in the shells it was not possible to determine the precise location of the boron. Normally, the boron in calcareous sediments is assumed to be in the detrital fraction, a correction factor being applied to determine the palaeosalinity<sup>13</sup>. However, the acid-insoluble fraction (composed entirely of clay-size material) proved to constitute on average no more than 1% by weight of the oyster shells (Table 1). If the mean boron content of non-calcareous clays is taken to be 160 p.p.m. (ref. 6), the acid-insoluble fraction will provide less than 2 p.p.m., or 10-15% of the total boron content of the shells. Also, there is no significant correlation between fraction of acid-insoluble material and the boron content. It therefore, seems most likely that the boron in oyster shells is mainly present within the calcite lattice, probably substituting for carbon. As chemical and X-ray diffraction results indicate that calcium carbonate is not preferentially leached from the shells, it would seem that substituted boron is being preferentially removed from the calcite lattice in oyster shells during the course of sub-aerial weathering. This might take place if the outer portion of the shells was boron rich but there is no reason to suspect that there is any such preferred location. Therefore, for the present it is only possible to point to the loss of boron during weathering without positively identifying the mechanism by which it is removed.

Returning then to the original point regarding the use of boron as an indicator of palaeosalinity. The Broad Sound results indicate that over a period of about 5,000 yr the boron content of oyster shells is considerably reduced. The extent to which these results from oyster shells may be extrapolated to other shells, and in turn to carbonate rocks in general, is uncertain. The results, however, undoubtedly suggest that errors may occur when taking the whole-rock boron content of carbonate rocks as a reflection of the original salinity, if those rocks have been subjected to sub-aerial weathering. It can perhaps be argued that where the boron content of the clay mineral only is considered that the problem of boron mobility is unlikely to arise. The Broad Sound study provides no information on the mobility of boron from clays. However, where the clay is in close proximity to shelly beds, and perhaps calcareous sediments in general, boron mobilised as a result of weathering is likely to be absorbed by the clay minerals. Where there is abundant shelly material this could conceivably result in a marked increase in the boron content of the associated clays, when compared with the original value. Further studies are necessary to fully assess whether the mobility of boron indicated by the Broad Sound study can be extended to calcareous and other sediments, and whether any such mobility limits the application of the boron method for the determination of palaeosalinity.

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## Pre-melting near crystal dislocations

IDEALLY, the melting of a crystalline solid should occur at a single temperature. In reality, the process is spread over a small range of temperatures. As the temperature approaches the nominal melting point  $T_m$ , the specific heat of, say, bismuth<sup>1,2</sup> acquires a component of the form

$$\Delta C = A/(T_m - T)^2 \quad (1)$$

where  $A$  denotes a constant for a given sample. Usually the premonitory effect is attributed to the presence of impurities that dissolve only in the liquid phase. Indeed, an expression close to equation (1) can be derived on this basis. There is, however, good evidence<sup>3</sup> to indicate that the effect cannot be wholly due to Raoult impurities. In the present letter, we want to show that equation (1) also characterises pre-melting near point defects, while that occurring along edge dislocations is described by

$$\Delta C = B/(T_m - T)^3 \quad (2)$$

where  $B$  denotes another constant.

Slater<sup>4</sup> in his classic paper on the ferroelectric transition of potassium dihydrogen phosphate indicates how a first-order transformation might acquire a temperature broadness at the hands of crystal imperfections. The same mechanism is invoked here to explain pre-melting. According to Slater, the stresses around crystal imperfections alter the effective pressure on



the various crystallites and thereby cause them to undergo the transition over a small temperature interval.

To treat pre-melting formally, we can use the continuum model of solids. For mathematical simplicity we take the material to be elastically isotropic. Thus the effective pressure around a point defect varies inversely as the cube of the distance, while that near an edge dislocation goes inversely as the distance. Further, let us assume that the difference in the densities of the two phases is sufficiently small so that any stresses resulting from this difference can be neglected. Since transition curves are practically straight lines, the volume of a liquid domain developing around a point defect should go as  $(T_m - T)^{-1}$  while that of a domain growing alongside an edge dislocation should be proportional to  $(T_m - T)^{-2}$ . Here we identify  $T_m$  as the temperature at which a crystallite subject to an effective pressure of 1 atm melts. Finally, using the fact that the change of entropy is proportional to the volume of the domains, we obtain for growth around point defects

$$\Delta C \propto T/(T_m - T)^2 \quad (3)$$

and for development along dislocations

$$\Delta C \propto T/(T_m - T)^3 \quad (4)$$

For most solids, the specific heat is anomalous over a temperature interval that is very small compared with the absolute temperature of melting. So relations (3) and (4) are virtually equivalent to equations (1) and (2), respectively.

The present findings lend themselves to a simple experimental test. First the anomalous component of the specific heat of a relatively pure bismuth crystal is determined. Then the crystal is severely deformed and the measurement is repeated. If sufficient dislocations are introduced, then the pseudo-critical exponent of its anomalous specific heat should go from  $-2$  to  $-3$ .

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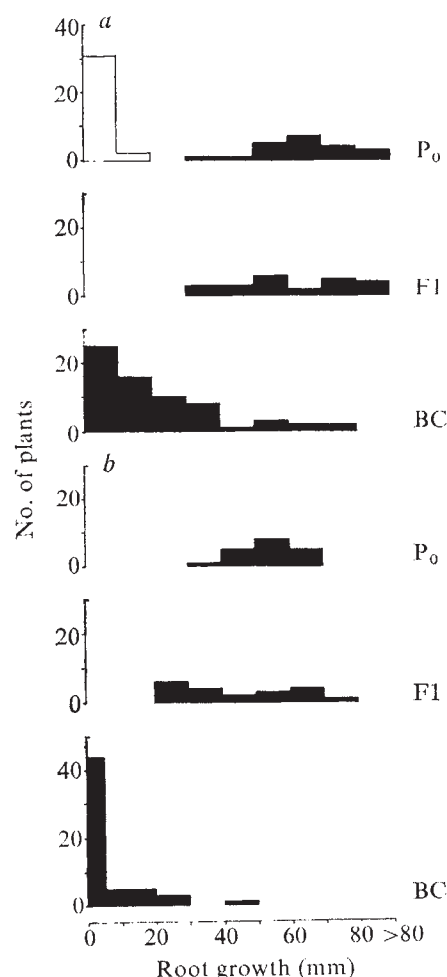
## Major genes for copper tolerance in *Mimulus guttatus*

HEAVY metal tolerance in higher plants has proved to be an excellent model system which has provided insight into several aspects of the evolutionary process (see ref. 1 for review). But comparatively little is known about the formal genetics of this character in higher plants, since the grasses which have been the subject of most of the ecological work are not readily amenable to genetic analysis. Antonovics *et al.*<sup>1</sup> argue that the data indicate that tolerance is governed by a polygenic system, though it has been shown that in yeast<sup>2,3</sup> and *Paramecium*<sup>4</sup> that major genes are responsible. The discovery<sup>5</sup> that the yellow monkey flower, *Mimulus guttatus*, has also developed copper tolerant races has enabled this hypothesis to be tested—the genetics of *M. guttatus* are readily investigated since it is easily destamated and crossed, produces up to 400 seeds at a time, and can complete a generation in less than 100 d. The data presented here suggest that there are only two genes responsible for most of the tolerance observed in this plant.

A single plant (C10), typical of one of the most tolerant

Californian populations, Copperopolis 4C, was crossed with a plant from the non-tolerant population at Cerig-y-drudion (for details of the populations see ref. 5). Forty F1 plants were tested for copper tolerance, and the most and least tolerant plants were then backcrossed to a Cerig plant. Twelve individuals of each of the F1 and the two backcross progenies were then grown in each of two fully randomised blocks. C10 was cloned, and 15 ramets of this plant and 15 Cerig individuals were also included in the randomisation. Because of the accidental loss of many of the plants from one block, a third block was later raised with 20 of each family. The data from all three blocks was pooled. Thus each family is represented by a maximum of 44 individuals. But, due to the death of some plants, and the fact that many individuals could not be tested at both levels of copper used in this experiment, the number of plants tested at any level is less than this.

The most immediate effect of heavy metals on non-tolerant plants is the inhibition of root growth<sup>6</sup>. This phenomenon provides the basis for the method of testing tolerance. Usually<sup>7,8</sup> an index is produced as the ratio of growth in a test solution to growth in a control solution, but in this instance, since we are concerned with the progeny of a single plant, the mean root growth of the



**Fig. 1** Histograms of rootgrowth of P<sub>0</sub> (C10 filled, Cerig open), F1 and combined backcross (BC) progenies at *a*, 0.125 and *b*, 0.5 p.p.m. copper. Cerig plants were only tested at 0.125 p.p.m. since they all produce roots shorter than 2 mm at the higher level. The root growth of each plant is the mean of two cuttings grown for a week in a solution of 0.5% (w/v) Ca(NO<sub>3</sub>)<sub>2</sub> containing the requisite level of copper as CuSO<sub>4</sub>. Solutions were changed on the third and fifth days. Testing was performed at 23 ± 1 °C with constant illumination. Note that not all plants could be tested at both levels. Full experimental details are given in ref. 9.

longest roots of two cuttings in a solution containing either 0.125 or 0.5 p.p.m. copper is used as the measure of tolerance. This method has the advantage that non-tolerant are more easily distinguished since at 0.125 p.p.m. they form short stumpy roots, while at 0.5 p.p.m. any roots which are produced die almost immediately.

Figure 1 gives the distribution of tolerance of the two parents, their F1 and the two backcross progenies, which have been pooled since they did not differ significantly (see Table 1b). At 0.125 p.p.m. it is apparent that the backcrosses contain many non-tolerant plants, but further conclusions at this level are limited by the large error inherent in measuring tolerance by this method. This error is revealed in Table 1, which gives the analysis of variance for each family at 0.125 p.p.m. More than 50% of the total variance of each of C10, Cerig and the F1 is due to measurement error (within plant variance). This variation is due to the natural variability of root growth: the principal source seems to be variation in the day of initiation (unpublished), though other factors are probably important (for example see ref. 10). Considerable genetic segregation in the backcrosses is indicated by the far larger proportion of its variance due to differences between individuals. Table 1 also shows that the C10, Cerig and F1 plants do not seem to be uniform at this level. The C10 ramets are genetically identical, and this variation is inexplicable in this instance. It is not found at other levels of copper in this clone, nor in two other clones tested. Significant differences within the Cerig population and the F1 family indicate that not all the individuals in these families are identical. In the case of the F1 this may indicate that C10 was heterozygous for some loci, but the lack of significant difference between the two backcross families indicates that this segregation is minor compared with the segregation in the backcrosses of loci homozygous in C10.

But at 0.5 p.p.m. Cerig plants are unable to form roots. Any that start to grow turn dark brown and die almost immediately. Any plant that shows any significant root growth must therefore be tolerant to a degree. The large error involved in tolerance testing is no longer so important because at this level we can make a qualitative distinction between tolerant (those plants which form roots) and non-tolerant (those that do not). All F1 plants are tolerant, but the backcrosses are segregating a large number of plants which effectively fail to root.

On this basis, the number of tolerant plants is 14, while there are 44 plants which are non-tolerant at this level. This ratio differs significantly from 1:1 ( $\chi^2 = 15.5$ ), but not from 1:3 ( $\chi^2 = 0.02$ ). A one gene model anticipates two equally frequent classes of progeny in the backcross, while a two locus model predicts three (AaBb; Aabb and aaBb; and aabb) in a 1:2:1 ratio, or 1:3 if the latter two classes cannot be distinguished. The data thus concord with a two-

locus model, but not with a single gene hypothesis. The data at 0.125 p.p.m. can also be explained on a two locus model. Figure 2 shows the relationship between the tolerances at high and low levels of copper of plants tested at both levels. It is evident that, despite the high variation in testing, the correlation between tolerance at 0.125 p.p.m. and 0.5 p.p.m. is high. The plants which prove to be tolerant at 0.5 p.p.m. are mostly the plants with the highest tolerances at 0.125 p.p.m. But, there are still many plants which show greater root growth than Cerig plants at 0.125 p.p.m. but which show none at 0.5. On a two-gene hypothesis, in

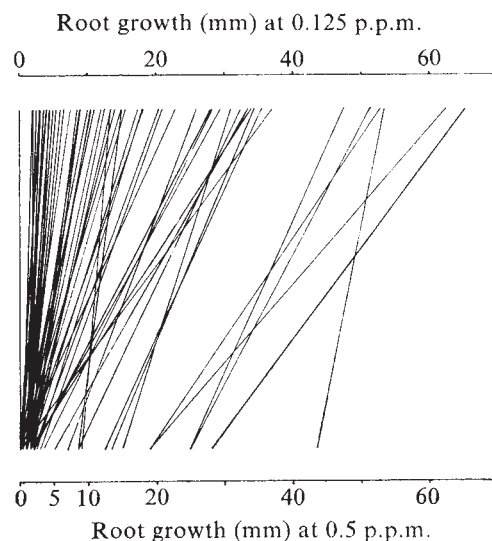


Fig. 2 The correlation between rootgrowth at 0.125 and 0.5 p.p.m. of the combined backcross progenies. All plants tested at both levels are included.

which only plants with both genes are tolerant, there is the problem of what the phenotype of a plant with only one gene is. These data would suggest that it could be partially tolerant at low levels of copper, but non-tolerant at higher levels.

Models involving more than two genes of roughly equal effect would need to be complicated in order to fit these data. With three genes there are four classes with 0, 1, 2 and 3 tolerance genes respectively, and these are expected in a 1:3:3:1 ratio: it is difficult to see how the data can be explained on such a segregation. It is also apparent that at 0.125 p.p.m. there are many plants which are as non-tolerant as Cerig plants: the segregation of plants with no tolerance genes at all becomes progressively rarer as the number of genes postulated is increased.

The data presented here indicate that there are two major

Table 1 Analysis of variance at 0.125 p.p.m. of a, C10, Cerig and the F1, and b, the backcross families

| Source             | d.f. | C10<br>MS | F      | d.f. | Cerig<br>MS | F     | d.f. | F1<br>MS | F     |
|--------------------|------|-----------|--------|------|-------------|-------|------|----------|-------|
| <b>a</b>           |      |           |        |      |             |       |      |          |       |
| Between plants     | 18   | 420.85    | 2.79*  | 32   | 19.51       | 2.06* | 22   | 731.41   | 2.62* |
| Within plants      | 19   | 150.68    |        | 33   | 9.45        |       | 23   | 279.50   |       |
| Error MS (%)       |      | 53%       |        |      | 65%         |       |      | 55%      |       |
| Total MS           |      |           |        |      |             |       |      |          |       |
| <b>b</b>           |      |           |        |      |             |       |      |          |       |
| Backcross families |      |           |        |      |             |       |      |          |       |
| Between families   | 1    | 22.46     | 0.03NS |      |             |       |      |          |       |
| Between plants     | 64   | 748.21    | 7.70†  |      |             |       |      |          |       |
| Within plants      | 66   | 97.11     |        |      |             |       |      |          |       |
| Error MS (%)       |      | 23%       |        |      |             |       |      |          |       |
| Total MS           |      |           |        |      |             |       |      |          |       |

In the case of C10, the different plants are all ramets. In all the other families, the different plants are all different individuals. d.f., Degrees of freedom; MS, mean square; F, variance ratio.

\*Significant at the 5% level.

†Significant at the 0.1% level.



genes involved in the production of copper tolerance in the Copperopolis population of this species. This conclusion is being tested by further breeding of the backcross progeny. However, these data do not rule out the possibility of there being other loci of lesser effect in addition. That this is likely is shown by the fact that crosses within the Copperopolis population reveal genetic variation<sup>9</sup>. All the Copperopolis plants tested were tolerant at 1.0 p.p.m., however, indicating that they were all homozygous for the two major genes.

This result has important physiological implications. Early work<sup>11-13</sup> on the physiology of tolerance suggested that zinc was bound up in the cell walls of zinc tolerant plants, thus preventing the metal from inhibiting cellular enzyme systems. Wainwright and Woolhouse<sup>14</sup>, however, argue that this may not be the only mechanism, and showed that individual enzyme systems may show tolerance. These results were not repeated in studies on different systems<sup>15,16</sup>. If enzymatic adaptation were a major component of tolerance one would expect that there would be many genes involved in producing it, because heavy metals are general protein inhibitors. Unless there is one enzyme system which is much more susceptible to copper than others, the substitution of a single non-tolerant enzyme by a tolerant form is unlikely to increase the tolerance of the whole phenotype by a large amount. A compound that was able to bind heavy metals and reduce their effective cellular concentration could make a significant contribution to the overall tolerance of an individual, however. It is possible that the production of such a compound could be mediated by a few genes. Thus the finding that there seem to be only two genes in *M. guttatus* producing most of the tolerance observed in the highly tolerant Copperopolis population tends to implicate a primary physiological mechanism involving the production of a protein able to bind copper and render it unavailable to the cell, rather than a system of general enzymatic adaptation.

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## Association of alleles at esterase loci in wild barley *Hordeum spontaneum* L.

THE aim of the conservation of plant genetic resources is to collect and preserve representative samples of genetic variation from the primitive 'landraces' and the related wild species of crop plants on which man depends for use in future breeding programmes<sup>1,2</sup>. Counter proposals<sup>3</sup> that breeders obtain specific alleles by mutagenesis at will are defensible when one is considering single loci. But groups of alleles at many loci which have jointly been subjected to natural selection, are unlikely to be assembled by mutation breeding. We report here the

widespread occurrence of co-adapted combinations of alleles at the four esterase loci in populations of wild barley (*Hordeum spontaneum*) in Israel, thus reinforcing the case for genetic conservation.

Cultivated barley (*Hordeum vulgare*) is polymorphic for four esterase loci. Three of the loci, denoted *Est A*, *Est B* and *Est C* are tightly linked ( $B \leftarrow 0.0023 \rightarrow A \leftarrow 0.0048 \rightarrow C$ ) and a fourth, *Est D* recombines independently<sup>4</sup>. In composite cross V, Allard and co-workers have described changes in gene frequency at these loci<sup>5</sup>, and have shown that only a few specific combinations of alleles were favoured<sup>6</sup>. Similarly in composite cross II, two balanced arrays account for 33% of the gamete pool<sup>7</sup> after 26 generations. These esterase loci are also highly polymorphic among the accessions in the US Department of Agriculture barley collection<sup>8</sup> of the ancestral species (*Hordeum spontaneum*) (ref. 9). Here we report that natural populations of wild barley in Israel also exhibit only a restricted number of complementary arrays of alleles at the esterase loci.

Single seedlings from about 50 panicles sampled from each of 11 populations were subjected to starch gel electrophoresis. The populations form transects running east to west (numbered 1-8 in Table 1), and north to south (9-11) across Israel. All loci were scored in a leaf sample, and *Est A* and *Est C* were scored in a separate root sample. *Est D* was scored in only three populations. The alleles were assigned to mobility classes with respect to the commercial cultivars Atlas (SMFM), and Clipper (MMFS). (SMFM and MMFS refer to slow (S), medium (M) and fast (F) 'mobility classes' of esterases specified by loci *A*, *B*, *C* and *D* respectively when subjected to starch gel electrophoresis.) Table 1 lists the frequencies ( $f_{ijk}$ ) of the two most common gametic arrays in each population. Thus, for example the 60% of the gametes in the Damun population were FSFM, 16% were MSMF and the remainder carried less frequent arrays. The observed number of alleles at each locus and the information at locus *X*

$$(I_X = -\sum_j p_j \log_2 p_j)$$

(ref. 10) are listed. Most populations are appreciably polymorphic. However, the observed average heterozygosity was less than 0.01, because the outcrossing rate ( $t$ ) was low ( $0 < t < 0.02$ ).

Table 1 also lists estimates of two and three locus measures of allelic association at the specified esterase loci. These logarithmic measures are computed as  $I_{XY} = \sum_{ij} \log_2(f_{ij}/p_i p_j)$  ( $f_{ij} > 0$ ) for two loci, and  $I_{XYZ} = \sum_{ijk} \log_2(f_{ijk}/p_i p_j p_k)$ , ( $f_{ijk} > 0$ ) for three loci<sup>11,12</sup>. There is a very high level of complementary association. For example, at Damun  $I_{AC} \approx \min(I_A, I_C)$  which is the theoretical maximum association in this case. Also  $I_{ACD} \approx I_{AC} + I_{CD}$  which is its theoretical maximum given the two locus values. The quantity  $(1.38 NI)$  where  $N$  is the number of independent gametes in the sample ( $N \approx 50$ ), is distributed asymptotically as  $\chi^2$  with appropriate degrees of freedom. The values of  $I$  can be tested for departure from zero or lack of association. Virtually all tests indicated statistically significant association. Intense association between alleles over loci have been reported in natural Californian populations of wild oats<sup>13</sup>. There is also significant association between esterase loci in animals (salamanders<sup>14</sup> and *Drosophila montana*<sup>15</sup>).

The data from wild barley presented here highlight two important difficulties of interpretation which cannot be resolved unequivocally. First, there is disagreement concerning the extent to which allozyme polymorphisms are maintained by natural selection<sup>16</sup>. Furthermore, it is recognised in addressing this question, that the loci cannot be considered separately from the chromosomal segments to which they belong. Second, when there is total self-fertilisation, selection is not necessary to preserve associations of alleles between loci, since this mating system is sufficient to retain any associations generated sporadically by mutation or random drift and partial isolation<sup>17</sup>.

**Table 1** Esterase polymorphism in eleven population of *Hordeum spontaneum*

| Population |            | Most common gametes and their frequency ( $f_{ijk}$ ) | Number of alleles and information ( $I$ ) at esterase loci |      |      |      | Two and three locus measures of association for specified combinations of esterase loci |    |        |
|------------|------------|-------------------------------------------------------|------------------------------------------------------------|------|------|------|-----------------------------------------------------------------------------------------|----|--------|
|            |            |                                                       | A                                                          | B    | C    | D    |                                                                                         |    |        |
| 1          | Akhziv     | Coastal                                               | MFS 0.42                                                   | 1    | 4    | 3    |                                                                                         | BC | 0.97** |
|            |            |                                                       | MMF 0.21                                                   | 0    | 1.70 | 1.36 |                                                                                         |    |        |
| 2          | Maalot     | Montane                                               | MFS 0.88                                                   | 1    | 4    | 3    |                                                                                         | BC | 0.57** |
|            |            |                                                       | MVF 0.06                                                   | 0    | 0.7  | 0.57 |                                                                                         |    |        |
| 3          | Mt Meron   | Montane                                               | MFS 1.00                                                   | 1    | 1    | 1    |                                                                                         |    |        |
|            |            |                                                       |                                                            | 0    | 0    | 0    |                                                                                         |    |        |
| 4          | Zefat      | Montane                                               | MFS 0.69                                                   | 1    | 4    | 3    |                                                                                         | BC | 0.84** |
|            |            |                                                       | MSM 0.20                                                   | 0    | 1.16 | 1.00 |                                                                                         |    |        |
| 5          | Tel Hai    | Foothills                                             | MVS 0.59                                                   | 1    | 3    | 2    |                                                                                         | BC | 0.98** |
|            |            |                                                       | MSM 0.29                                                   | 0    | 1.33 | 0.98 |                                                                                         |    |        |
| 6          | Rosh Pinna | Foothills                                             | MPM 0.66                                                   | 2    | 4    | 2    |                                                                                         | AB | 0.66** |
|            |            |                                                       | FIM 0.17                                                   | 0.66 | 1.38 | 0.66 |                                                                                         | AC | 0.05   |
| 7          | Gadot      | Rift Valley                                           | MIM 0.27                                                   | 4    | 5    | 3    |                                                                                         | AB | 0.39** |
|            |            |                                                       | MVS 0.23                                                   | 0.77 | 2.30 | 1.51 |                                                                                         | AC | 0.05   |
| 8          | Shifon     | Montane                                               | MMS 0.85                                                   | 2    | 2    | 1    |                                                                                         | AB | 0.01   |
|            |            |                                                       | MVS 0.10                                                   | 0.19 | 0.48 | 0    |                                                                                         |    |        |
| 9          | Damun      | Montane                                               | FSFM 0.60                                                  | 2    | 1    | 3    | 3                                                                                       | AC | 0.90** |
|            |            |                                                       | MSMF 0.16                                                  | 0.90 | 0    | 1.21 | 1.12                                                                                    | AD | 0.25** |
| 10         | Bar-Giora  | Montane                                               | MFMM 0.44                                                  | 2    | 3    | 2    | 1                                                                                       | AB | 0.48** |
|            |            |                                                       | MMSM 0.46                                                  | 0.66 | 1.38 | 0.99 | 0                                                                                       | AC | 0.10*  |
| 11         | Yeruham    | Steppe                                                | MMVF 0.86                                                  | 1    | 1    | 3    | 3                                                                                       | CD | 0.26** |
|            |            |                                                       | MMSM 0.04                                                  | 0    | 0    | 0.47 | 0.82                                                                                    |    |        |

\*Association significant at the 5% level.

\*\*Association significant at the 1% level.

However, provided there is some outcrossing and recombination, strong associations cannot persist in equilibrium populations if the chromosomal segments are neutral to selection<sup>18,19</sup>. We conclude that the occurrence of allelic associations for marker loci in natural populations of the wild relative of a crop plant is an observation crucial to the rationale of genetic conservation.

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## Prediction of seed longevity at sub-zero temperatures and genetic resources conservation

THE International Board for Plant Genetic Resources (IBPGR) is encouraging the development of an international network of seed banks for the conservation of the genetic resources of the major crop species of the world and their wild relatives<sup>1</sup>. Most of the species in priority lists show 'orthodox' seed characteristics<sup>2</sup>, that is their longevity is prolonged in a defined manner by a reduction in moisture content (MC) (at least down to 5% MC, fresh weight basis) and temperature<sup>3</sup>. From a consideration of biological factors and the design and cost of seed storage facilities, IBPGR recommends that orthodox seeds are stored at  $-18^{\circ}\text{C}$  or less and at  $5 \pm 1\%$  MC<sup>2</sup>. It is convenient to aim at  $-20^{\circ}\text{C}$  as the nominal temperature. Because of the accumulation of mutations associated with loss of viability<sup>4,5</sup>, IBPGR recommends regeneration of stocks (that is growing plants to provide new seeds from the old) when viability has fallen by 5% (ref. 2). To plan ancillary facilities and procedures, it is necessary to estimate regeneration intervals (time taken for viability to fall by 5%) for the major crop species. Such estimates pose three problems: there are genotypically controlled variations in longevity within a species; seed 'quality', determined by conditions before harvest and during processing, can affect subsequent longevity; and loss of viability at  $-20^{\circ}\text{C}$  is extremely slow, and so far it has been possible to predict regeneration interval only by questionable extrapolation from more favourable storage conditions<sup>5,6</sup>. We have examined these problems in barley (*Hordeum distichum*, L.) and have developed an approach which should make possible more accurate predictions from experiments lasting only 2 yr—even though fresh seed of most species shows no detectable loss of viability during this time.

Loss of viability in orthodox seeds conforms closely to three basic viability equations applicable over a wide range of conditions in all seven species that have been examined in detail (Table 1) as follows<sup>3,6</sup>. The frequency of individual deaths in time in a population stored in constant conditions is described by the normal distribution



$$y = \{1/\sigma\sqrt{(2\pi)}\} \exp\{-(p-\bar{p})^2/2\sigma^2\} \quad (1)$$

where  $y$  is the relative frequency of deaths occurring at time  $p$ ,  $\bar{p}$  is the mean viability period, and  $\sigma$  is the standard deviation of the distribution of deaths in time. Consequently survival curves (percentage viability plotted against time) are cumulative normal distributions of negative slope, which become straight lines if transformed to probit (as in Figs 1 and 2). The spread of the distribution is directly proportional to the mean viability period, that is

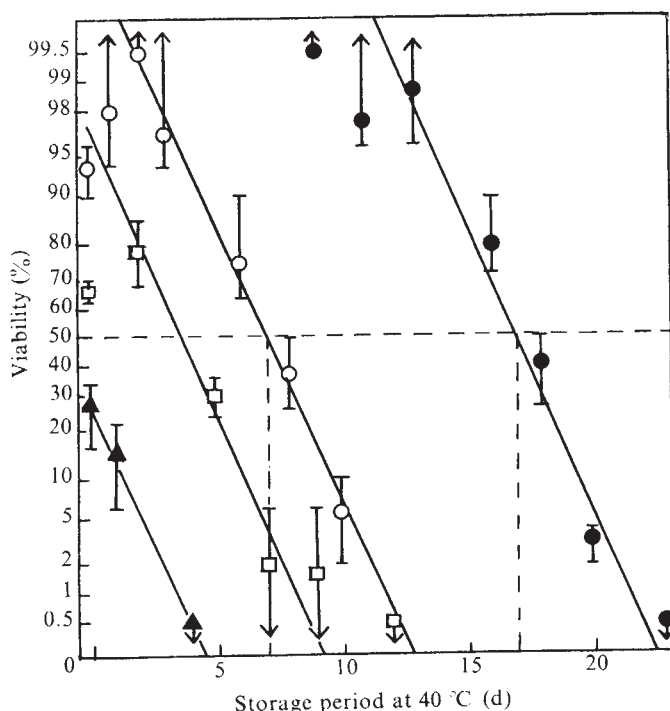
$$\sigma = K_\sigma \bar{p} \quad (2)$$

where  $K_\sigma$  is a constant. And the relationship between moisture content, temperature and mean viability period is described by the equation

$$\log \bar{p} = K_v - C_1 m - C_2 t \quad (3)$$

where  $m$  is moisture content (% fresh weight basis),  $t$  is temperature ( $^{\circ}\text{C}$ ), and  $K_v$ ,  $C_1$  and  $C_2$  are constants. Thus once the four viability constants ( $K_v$ ,  $K_\sigma$ ,  $C_1$  and  $C_2$ ) have been determined it is possible to define percentage viability after any period of storage in any combination of conditions.

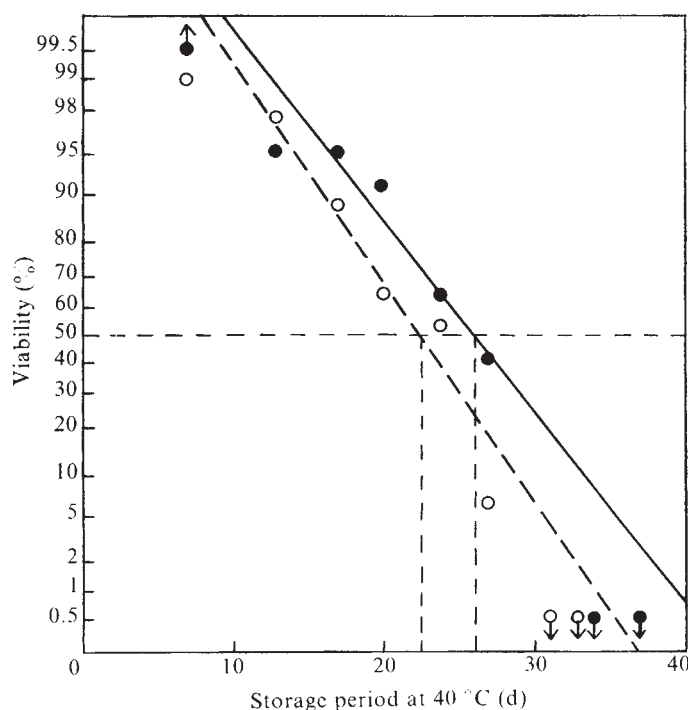
We have examined five cultivars of barley (Vada, Berac, Julia, Golden Promise and Proctor) in various storage treatments and have found that the  $C$  constants have identical values ( $C_1 = 0.098$  and  $C_2 = 0.050$ ). Genotypic differences in longevity are attributable to differences in the value of the  $K$  constants:  $K_\sigma$  varies only slightly (mean value 0.214), but



**Fig. 1** Survival curves of barley seed (*H. distichum* cultivar Proctor) at 15.4% moisture content in hermetic storage at 40  $^{\circ}\text{C}$  after 0 (●), 3 (○), 4 (□), and 5 (▲) d previous storage at 50  $^{\circ}\text{C}$ . Percentage viability (mean of five replicates of 50 seeds each) is plotted on a probability scale. The vertical bars represent the extreme values of the replicates. Since 100% and 0% cannot be shown on a probability scale, such values are represented by arrows pointing upwards at 99.5% or downwards at 0.5%. Germination tests to determine viability were carried out at 7 d at 3  $^{\circ}\text{C}$  (to remove dormancy) followed by 14 d at 20  $^{\circ}\text{C}$ . Extension of the radicle by  $> 2$  mm was taken to indicate a viable seed. Note that 3 d of preliminary storage at 50  $^{\circ}\text{C}$  had no significant effect on initial percentage viability, but decreased the mean viability period from 17 to 7 d.

there are quite large differences in  $K_v$ , varying from 4.69 (when  $\bar{p}$  is expressed in days) in Julia, to 5.56 in Proctor. Since  $K_v$  is related to  $\log \bar{p}$ , this difference results in a sevenfold variation in longevity between the cultivars in identical storage conditions and, because the values of the  $C$  constants are identical, it follows from equation (3) that this relative difference is maintained in all storage conditions.

In experiments using Proctor we have investigated treatments on the seed crop which could affect subsequent viability, including nitrogen nutrition (15 or 70 kg ha $^{-1}$  N), shading (intercepting 60% radiation for a month before harvest), degree of unripeness, and severity of threshing. Only shading and marked unripeness significantly affected longevity. As with genotypic effects, only the  $K$  constants were affected ( $K_\sigma$  only slightly).  $K_v$  was reduced from 5.56 to 5.54 by shading and to 4.70 by harvesting unripe seed. Thus only unripeness had a major effect, but this was as much as could be caused by genotypic effects. Now that it is known that both genotypic and prestorage environmental factors only have any practical effect on  $K_v$ , further estimates of possible variation in longevity within a species are greatly simplified.



**Fig. 2** The effect of 750 d of hermetic storage at  $-20^{\circ}\text{C}$  and 15.4% moisture content on the viability of barley seeds (*H. distichum* cultivar Proctor). Seed at 15.4% moisture content was hermetically stored at 40  $^{\circ}\text{C}$ . Paired samples were withdrawn at intervals and viability immediately determined on one sample (●). The second sample in each case was stored for a further 750 d at  $-20^{\circ}\text{C}$  after which viability was again determined (○). Each point represents the mean of four replicates of 50 seeds. Further explanation as for Fig. 1.

To develop a technique for estimating the very slow rates of deterioration which occur at sub-zero temperatures, it first had to be confirmed, using alternating temperature regimes, that change in temperature *per se* had no effect on longevity. It was then established that any preliminary seed deterioration did not affect the subsequent slope of the survival curve, but only the intercept: slope is determined solely by contemporary conditions (Fig. 1). Figure 2 shows that although 750 d of storage at  $-20^{\circ}\text{C}$  had no significant effect on initial viability, as would be expected from the shape of the survival curve, there is a maximum affect on percentage germination on samples approaching 50% viability. From the  $\bar{p}$  values shown by the vertical broken lines, it can be calculated that 750 d of

**Table 1** Provisional estimates of probable regeneration intervals for seeds stored at  $-20^{\circ}\text{C}$  and 5% moisture content

| Species                             | Cultivar                     | Viability constants* |       |       |       | Probable regeneration interval (yr)† |
|-------------------------------------|------------------------------|----------------------|-------|-------|-------|--------------------------------------|
|                                     |                              | $K_0$                | $K_v$ | $C_1$ | $C_2$ |                                      |
| Barley ( <i>Hordeum distichum</i> ) | Proctor, Golden              | 0.214                | 4.800 | 0.098 | 0.050 | 70                                   |
|                                     | Promise, Julia (mean values) |                      |       |       |       |                                      |
| Rice ( <i>Oryza sativa</i> )        | Norin                        | 0.200                | 5.680 | 0.150 | 0.047 | 300                                  |
| Wheat ( <i>Triticum aestivum</i> )  | Atle                         | 0.350                | 5.067 | 0.108 | 0.050 | 78                                   |
| Broad bean ( <i>Vicia faba</i> )    | Claudia supraquadrulce       | 0.379                | 5.766 | 0.139 | 0.056 | 270                                  |
| Pea ( <i>Pisum sativum</i> )        | Meteor                       | 0.384                | 6.432 | 0.158 | 0.065 | 1,090                                |
| Onion ( <i>Allium cepa</i> )        | White Portugal               | 0.486                | 4.906 | 0.107 | 0.055 | 28                                   |
| Lettuce ( <i>Lactuca sativa</i> )   | Grand rapids                 | 0.110                | 3.805 | 0.079 | 0.047 | 11                                   |

\*Sources of values for viability constants: barley<sup>7</sup>; rice, calculated from figures supplied by Dr H. Ito<sup>8</sup>; wheat<sup>9</sup>; broad bean and pea<sup>10</sup>; onion<sup>7</sup>; lettuce, Dr Ann Minchin (unpublished results).

†Probable regeneration interval is calculated as predicted time for viability to fall to 95% of initial value ( $p_{95}$  period). Because 95% of the area under a normal curve (that is 95% of seed deaths) occurs when storage period  $> \bar{p} - 1.645\sigma$ , it follows from equation (2) that  $p_{95} = \bar{p} - 1.645K_0\bar{p}$ . The value of  $\bar{p}$  was solved by inserting the appropriate viability constants in equation (3) at 5% MC and  $-6^{\circ}\text{C}$ . The value  $-6^{\circ}\text{C}$  rather than  $-20^{\circ}\text{C}$  was chosen on the basis of the preliminary work on barley which indicated that equation (3) underestimates rate of loss of seed viability at sub-zero temperatures.

storage at  $-20^{\circ}\text{C} \triangleq 3.8$  d of storage at  $40^{\circ}\text{C}$ , that is, 1 d at  $40^{\circ}\text{C} \triangleq 197$  d at  $-20^{\circ}\text{C}$ . If  $C_2$  has the same value at  $-20^{\circ}\text{C}$ , the predicted temperature,  $t_p$ , at which this result should have been obtained is given by  $t_p = 40 - (\log 197/C_2)$ , that is  $-6.3^{\circ}\text{C}$ . Thus it seems that the rate of deterioration at  $-20^{\circ}\text{C}$  was greater than would have been predicted by extrapolation from higher temperatures.

These results have suggested an improved technique which we believe should be applied to the major crop species to estimate regeneration interval. It depends on the strong evidence that the value of  $K_0$  remains the same in all storage conditions<sup>9,7</sup>. If a sample were reduced to about 50% viability by rapid ageing (for example, storage at  $40^{\circ}\text{C}$  at about 15% MC), it could then be stored at  $-20^{\circ}\text{C}$  for, say, 2 yr when significant losses of viability would be expected. Sequential germination test over this period would determine the slope of the survival curve from which  $\sigma$ ,  $\bar{p}$  and  $K_0$  could be calculated (equation (2)). Similar treatments at several other moisture contents and sub-zero temperatures would provide additional values of  $\bar{p}$ , making possible simultaneous solution of equation (3) and providing the values of all four viability constants determined in conditions approximating those recommended, and avoiding the need to extrapolate from higher temperatures.

Further work is needed, but will take a few years to complete. Meanwhile practical decisions have to be made, and it is suggested that at present best estimates of regeneration interval are obtained by applying the three viability equations and the four viability constants determined at higher temperature to predict the time taken for viability to fall by 5% at  $-6^{\circ}\text{C}$  (because extrapolation to  $-20^{\circ}\text{C}$  seems to underestimate the rate of deterioration at that temperature). Although such estimates (Table 1) may be far from perfect, they suggest that in the conditions recommended by IBPGR, seeds of many species are stored without regeneration for more than a century; but some species may need to be regenerated in less than 20 yr. Because longevity is affected by both genotype and pre-storage environment, it is essential that stocks are monitored for loss of viability at intervals which relate to expected longevity. It is not sufficient, however, to be guided entirely by the expected time for viability to fall by 5% ( $p_{95}$  period). The seed-to-seed variation in longevity (described by  $K_0$ ) which determines the slope of the survival curve is also important, because a species with a low  $K_0$  value will give relatively little warning of an impending fall in viability (for example, lettuce in Table 1), and such species should be monitored more frequently.

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## Night flight towards a sex pheromone source by male *Spodoptera littoralis* (Boisd.) (Lepidoptera, Noctuidae)

PHEROMONES have been used in population monitoring traps<sup>1</sup> and in control by 'confusion'<sup>2</sup>, and a good understanding of how the male finds the female is of great practical importance in the development of these techniques. The problem of insect orientation towards distant odour sources is also intrinsically interesting and much work has been done with wind tunnels to study the flight of moths in attractant plumes (the most recent of many reports is ref. 3). These studies have shown that moths fly upwind in a series of horizontal zig-zags which decrease in amplitude as the source is approached. Field observations support these general findings<sup>4</sup>, but they are not as detailed as the results of laboratory studies and the techniques are not suitable for night flying species, many of which have considerable economic importance. We describe here techniques for observing and recording the details of the behaviour of night flying insects in attractant plumes, and also some new findings which suggest that flight towards a sex pheromone source in one species consists of several distinct stages with deviations from a straight upwind path taking place largely in the vertical plane.

The work was carried out in Crete during August and September 1975 and 1976 as part of experiments on the use of sex pheromones to control *Spodoptera littoralis* (Boisd.), a noctuid moth, the larvae of which damage many crops, including lucerne and cotton. For flight trials the synthetic sex attractant pheromone *cis*-9, *trans*-11-tetradecadien-1-yl acetate<sup>5</sup> was used, dispensed from a cylindrical polythene vial 3 cm long and 1.5 cm in circular cross section diameter, giving a release rate of order  $5 \text{ ng min}^{-1}$ . This was suspended 1 m above the ground in lucerne 0.2–0.3 m high. Even in bright moonlight *S. littoralis* males cannot easily be seen beyond about 2 m and to see moths at greater distances a night-viewing device (NVD)



was used. This consists of a cascaded set of photomultiplier tubes, giving a light magnification of order  $\times 40,000$  (ref. 6) and optics which produce an image magnified  $\times 2.5$ . In field conditions a grey object of  $5 \text{ cm}^2$  can normally be seen at 25 m in bright starlight. The instrument has a very large object lens, however, and therefore a large effective aperture; at the desired ranges, of order 20 m, the depth of field is inadequate for small moving targets. Also the image at low light levels is coarse grained and scintillates strongly. Both of these limitations have been overcome by using supplementary infrared illumination which greatly improves image brightness and definition in the NVD and facilitates the use of aperture disks effectively to 'stop down' the object lens, thereby increasing depth of field. With a 4-mm aperture, the depth of field at 25 m is about 10 m. Two 55-W car spotlights were placed 16 m apart on the wind-line through the attractant source and shining towards each other and the source, with the upwind lamp some 6 m from the attractant. The beams were filtered by infrared gelatine filters (Wratten 87) which nominally pass light in the wavelength range 750–1,000 nm only, well beyond the sensitivity found in the eyes of most insects except at very high intensity<sup>7</sup>.

Flight behaviour was observed both from a position 10–20 m to the side of the expected flight path and from upwind of the source looking along the path. Portions of the flight path were recorded in side view by two methods. A 16-mm cine camera recorded the NVD image, and infrared-sensitive film was used in a 35-mm still camera with a sector shutter giving a series of images of the moth's flight on one frame. Figure 1 shows the last few metres of a flight as an example of the latter method,



Fig. 1 Multi-exposure infrared photograph showing 'undulating' and 'hovering' flight. Scale shown is approximately 1 m. Interval between successive frames was approximately  $1/30 \text{ s}$ . Wind was from the left.

and Fig. 2 shows a reconstruction of a flight path made by superimposing drawings of the moth's position taken from a series of consecutive frames of cine film. From the measured and scaled time interval, the actual speed of the insect along its flight path relative to the ground (the 'speed of flight') and its horizontal component, the ground speed, can be calculated. Both techniques make it possible to see the heading of the moths and on the 35-mm frames the body angle of attack can also be seen.

Male moths showed a surprisingly consistent flight pattern and in favourable conditions (a steady wind greater than  $0.25 \text{ m s}^{-1}$ ) distinct successive stages of flight were found, performed by some 90% of about 1,000 moths identified as *Spodoptera littoralis* on the strict criterion of their halting their forward progress on the pheromone capsule or in the air close to it. (Some insects of about the size of *S. littoralis* flew straight upwind past the capsule without any apparent recognition of it; it is possible that some of these may have been *S. littoralis* males, but they were discounted.)

Figure 3 shows the general flight pattern. In steady wind the moths were first seen in straight and level flight, making rapid

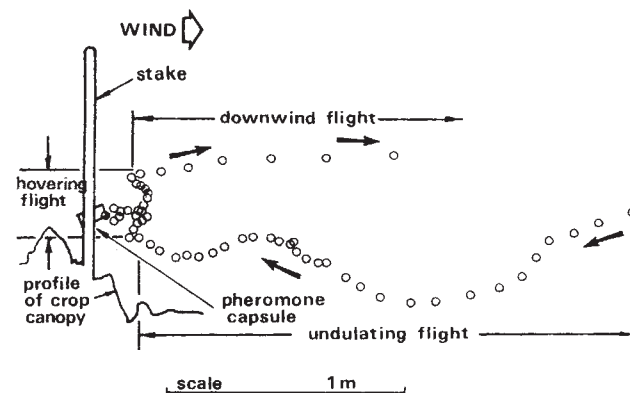
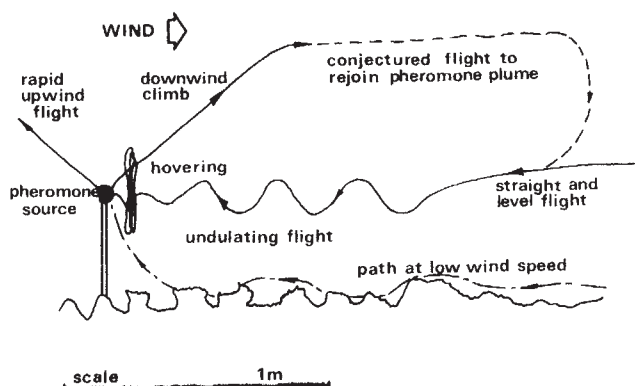


Fig. 2 Flight track produced from cine film. The circles represent the moth's position on consecutive frames.

progress directly upwind with an estimated ground speed of  $3 \text{ m s}^{-1}$  and at an altitude of between 0.5 m and 2.0 m; this portion of the flight was outside the field of view of the cameras. At a distance from the source usually within the range 2–4 m, and apparently fixed for a given wind speed, the moths abruptly reduced both ground speed and 'speed of flight', performing subsequently a slower, more undulating flight of about  $0.5 \text{ m s}^{-1}$ . This is shown in Fig. 1 up to about 0.5 m from the source and in Fig. 2 up to about 0.2 m. Although the flight path contained some deviations in the horizontal plane, well defined zig-zagging took place only as undulation in the vertical plane.

Closer to the source, forward progress was halted and vertical oscillations increased until the moth was hovering downwind from the source and moving up and down over a distance of up to 1 m while maintaining its upwind heading (Figs 1 and 2). Hovering could last up to 10 s during which occasional, less regular, short, downwind flights and general exploratory movements were made. About half of these moths then turned and flew rapidly downwind, often climbing to an altitude of order 2–3 m where they were usually lost from view. About half of them again landed near or on the capsule and moved about rapidly in a small area, often fanning with the wings. In Fig. 2 the moth very briefly touched the capsule before flying away in a gentle downwind climb accelerating to  $2.2 \text{ m s}^{-1}$  by the last frames. The moths which did land usually settled for up to about 1 min before leaving the capsule either downwind or in a rapid climbing upwind flight, again generally out of the illuminated region. On five nights moths were observed from a greater distance, 30–40 m, and it was then possible to see them climbing from the capsule and at an altitude of some few metres swiftly turning to head and fly downwind and then dropping to the original approach height about 10–20 m from the source. It was very difficult to see the moths after this because the image they produced in the NVD at such distance could be confused with

Fig. 3 Generalised flight pattern and the effect of low wind speed on it.



that produced by the lucerne leaves, but on two occasions it was possible to see them flying towards the attractant source again. At lower wind speeds the initial stages of the approach flight were made at very low altitude; in winds below about 0.1 m s<sup>-1</sup> moths flew within the crop canopy, from which they made the final approach in a climb which became steeper as wind speed became less.

The observations suggest that the flight of male *Spodoptera littoralis* to a pheromone source is made in discrete stages with different flight patterns and perhaps also different orientation mechanisms. The flight paths observed showed a strongly vertical undulation pattern which contrasts with the horizontal zig-zagging more generally seen. This is not understood, but it may be due in some way to differences in the structure of the pheromone plume.

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## Effect of regeneration on compartment specificity of the bithorax mutant of *Drosophila melanogaster*

In insect imaginal disks the basis for determination may be the state of a series of loci, each switched on or off in a given cell lineage at a unique point in development known as a compartmentalisation event<sup>1</sup>. Clones induced after the blastoderm stage define homologous anterior and posterior compartments in the thoracic disks<sup>2</sup>. Bithorax mutants transform the anterior compartments of the metathoracic disks (halter and third leg) into those of the mesothorax (wing and second leg); however, a wild-type allele must function continuously until metamorphosis to prevent the trans-

formation<sup>3,11</sup>. This suggests that permanent activation of the gene at blastoderm accounts for heritability of the determined state. Compartmental commitments are apparently lost when disk fragments regenerate<sup>4,5</sup>. We therefore studied duplication patterns in the third legs of a temperature-sensitive cell-lethal mutant also homozygous for bithorax. Heat treatment of these larvae kills cells, producing disk fragments which regenerate *in situ* giving duplicated appendages<sup>6-8</sup>. We report here that bithorax sometimes transforms posterior compartments of duplicated legs, showing that its effects are not invariably compartment-specific.

Duplicated and deficient (DFP) third legs from heat-treated 726; *bx<sup>34e</sup>/TM6* or 726; *bx<sup>34e</sup>/bx<sup>34e</sup>* individuals were scored for several morphological markers that differ between meso- and metathorax. 'Original' and 'duplicate' members of DFP legs could be distinguished on the basis of symmetry and size. Control data were collected by scoring unduplicated legs from the same flies and from a sample of *bx<sup>+</sup>* controls. The results are presented in Table 1. The *bx<sup>+</sup>* data demonstrate the reliability of the markers. The only marker that lacks complete specificity is the 'hairy island bristle' (BH<sup>-</sup>). This is invariably present in mesothoracic legs, but also appears in the metathorax, especially in *TM6* heterozygotes. The data from *bx<sup>34e</sup>* and *bx<sup>34e</sup>/TM6* flies should therefore be compared only with the appropriate controls when this marker is considered.

Among bithorax third legs we found only markers of the mesothorax in the anterior compartments. In the posterior compartments of unduplicated legs and original members of DFPs we found only metathoracic markers (rows 5-8 of Table 1). But, in the posterior compartments of duplicate members we found markers of both meso- and metathorax (rows 9 and 10).

These results clearly show (1) that the effect of bithorax is confined to the anterior compartment of the unduplicated third leg, as in the haltere, (2) that the penetrance of the *bx<sup>34e</sup>* allele is complete in the leg, and (3) that when the leg duplicates, the posterior compartment of the duplicate is sometimes also transformed. The transformation usually affects every marker in the compartment. Only 2 out of 50 cases are putative exceptions, each having a single discordant marker. Examples of the two major classes of duplication are illustrated in Fig. 1.

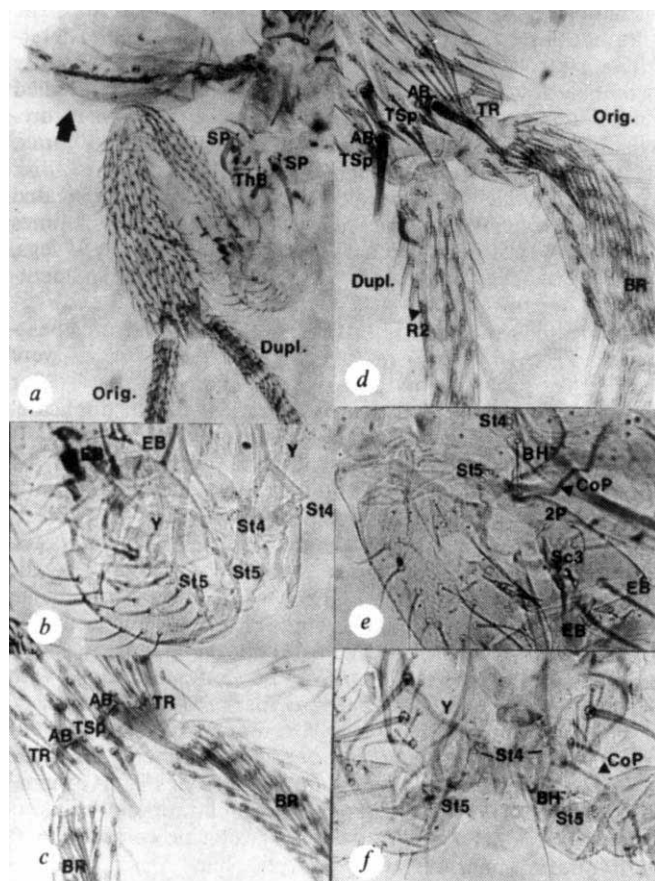
The posterior compartment of the duplicate was transformed to mesothorax in 18 out of 50 DFPs. The incidence of transformation was not significantly different in the two genotypes tested (Table 2a). The possibility that trans-

**Table 1** Number of times markers of meso- and metathoracic legs were present in samples of duplicated third legs and in controls

| Kind of leg         | Genotype                    | Number | Anterior meso- |            |           |           | Posterior meta- |           |           |           | Posterior meso- |                       |           |                       |           |
|---------------------|-----------------------------|--------|----------------|------------|-----------|-----------|-----------------|-----------|-----------|-----------|-----------------|-----------------------|-----------|-----------------------|-----------|
|                     |                             |        | <i>SP</i>      | <i>ThB</i> | <i>EB</i> | <i>AB</i> | <i>Y</i>        | <i>ID</i> | <i>TR</i> | <i>BR</i> | <i>P</i>        | <i>BH<sup>-</sup></i> | <i>2P</i> | <i>TS<sub>P</sub></i> | <i>R2</i> |
| Normal 2nd          | + +                         | 20     | 20             | 20         | 20        | 20        | 0               | 0         | 0         | 0         | 20              | 20                    | 20        | 20                    | 20        |
|                     | +/ <i>TM6</i>               | 20     | 20             | 20         | 20        | 20        | 0               | 0         | 0         | 0         | 20              | 19                    | 20        | 20                    | 20        |
| Normal 3rd          | +/+                         | 20     | 0              | 0          | 0         | 0         | 20              | 10        | 20        | 20        | 0               | 2                     | 0         | 0                     | 0         |
|                     | +/ <i>TM6</i>               | 20     | 0              | 0          | 0         | 0         | 20              | 9         | 20        | 20        | 0               | 11                    | 0         | 0                     | 0         |
|                     | <i>bx<sup>34e</sup></i>     | 40     | 40             | 40         | 40        | 40        | 40              | 8         | 40        | 40        | 0               | 0                     | 0         | 0                     | 0         |
|                     | <i>bx<sup>34e</sup>/TM6</i> | 44     | 44             | 44         | 44        | 44        | 44              | 22        | 44        | 44        | 0               | 13                    | 0         | 0                     | 0         |
| DFP 3rd (original)  | <i>bx<sup>34e</sup></i>     | 31     | 29             | 29         | 31        | 31        | 26              | 2         | 30        | 31        | 0               | 0                     | 0         | 0                     | 0         |
|                     | <i>bx<sup>34e</sup>/TM6</i> | 19     | 16             | 16         | 19        | 19        | 13              | 7         | 19        | 19        | 0               | 6                     | 0         | 0                     | 0         |
| DFP 3rd (duplicate) | <i>bx<sup>34e</sup></i>     | 31     | 27             | 27         | 30        | 29        | 16              | 0         | 19        | 22        | 6               | 4                     | 3         | 8                     | 10        |
|                     | <i>bx<sup>34e</sup>/TM6</i> | 19     | 16             | 16         | 17        | 17        | 9               | 1         | 10        | 10        | 3               | 5                     | 3         | 7                     | 7         |

Experimental flies were obtained by breeding from a *yw726*; *bx<sup>34e</sup>/TM6* stock. The symbol '726' refers to a temperature-sensitive lethal allele, *1(I)ts726*, at the suppressor-of-forked locus (1-65.9). When larvae carrying 726 are placed for 48 h at 29 °C, pattern deficiencies and duplications (DFPs) are formed in the surviving adults<sup>6</sup>. Cell-death creates disk fragments that duplicate during the extended larval period caused by heat treatment<sup>7</sup>. Leg deficiencies affect contiguous sets of markers located medially on the fate map<sup>8</sup>. *bx<sup>34e</sup>* is an allele at the bithorax locus (3-58.8). The third chromosome balancer *TM6* carries the markers *bx<sup>34e</sup>* and *Ubx<sup>67b</sup>* (Ultrabithorax; 3-58.8) which is lethal as a homozygote. Further details may be found in Lindsley and Grell<sup>9</sup>. Homozygous *bx<sup>34e</sup>* progeny were distinguished from *bx<sup>34e</sup>/TM6* heterozygotes by scoring the halteres which are much more strongly transformed when *TM6* is present. Parents were allowed to lay eggs for 5 d at 22 °C in bottles containing a standard medium<sup>6</sup>. The parents were discarded and the cultures shifted to 29 °C for 48 h then returned to 22 °C. On eclosion, adults of appropriate genotypes were collected and examined for duplications. Pharate adults that failed to eclose were dissected from the pupal case and treated similarly. Flies with duplications were dissected and mounted for detailed examination as described elsewhere<sup>8</sup>. Morphological markers were scored under the compound microscope. For details of markers used see Fig. 1 legend. Markers were assigned to compartment following Steiner (1976)<sup>2</sup>.





**Fig. 1** *a-c*, DFP third leg in a *bx<sup>34e</sup>/TM6* fly; *b* and *c* show details of markers in proximal and distal segments. Anterior haltere is transformed into wing (arrow). Anterior compartments of both original and duplicate parts of leg bear mesothoracic markers: sternopleurals (SP), thoracic bristles (ThB), edge bristles (EB) and apical bristles (AB). The posterior compartments of both elements bear the following metathoracic markers: the Y-shaped apodeme (Y), tibial transverse row (TR), basitarsal transverse rows (BR). *d-e*, DFP *bx<sup>34e</sup>* third leg. The duplicate posterior compartment is transformed into that of the second leg. In *d* note tibial spurs (Tsp) flanking the apical bristle on both sides in the duplicate, and on one side in the original. Duplicate also has a single row of bristles (R2) in the basitarsus where the original has transverse rows (BR). Coxa and trochanter are shown in *e*. Markers in the focal plane belong to the duplicate posterior compartment, and are characteristic of the mesothoracic leg. BH<sup>-</sup>, hairy island bristle; CoP, coxal process; 2P, bristles proximal to Sc3. St4, St5 and Sc3 are found in both meso- and metathoracic legs (compare Fig. 1*b*). *f*, DFP 3rd leg in which duplicate is mesothoracic. This example shows the Y-apodeme in the original leg (Y) with arms adjoining St4 and St5. This clearly is not present in the duplicate, which instead shows a hairy island bristle (BH<sup>-</sup>) and a coxal process (CoP).

formation depends on stage at heat treatment is ruled out by the fact that in four out of five cases in which both third legs from the same fly were duplicated, one was transformed, and the other was not. We also scored each DFP for a series of morphological markers common to meso- and metathoracic legs. Deficiencies for these markers may either fall entirely within the anterior compartment, or may also extend into the posterior<sup>8</sup>. Posterior cells might be expected to participate more often in the formation of the regeneration blastema in the latter event. Contrary to expectation, no significant difference in the frequency of transformation was found (Table 2*b*).

The following interpretation is tentative, but susceptible of verification. Transformation of the duplicate posterior

**Table 2** Incidence of transformation of posterior compartment of duplicate, classified by *a*, genotype and *b*, extent of deficiency

| Transformation       | <i>a</i> Genotype       |                             | <i>b</i> Deficiency |                        |
|----------------------|-------------------------|-----------------------------|---------------------|------------------------|
|                      | <i>bx<sup>34e</sup></i> | <i>bx<sup>34e</sup>/TM6</i> | Anterior only       | Anterior and Posterior |
| Anterior only        | 21                      | 11                          | 21                  | 11                     |
| Anterior + posterior | 10                      | 8                           | 13                  | 5                      |
| Contingency $\chi^2$ |                         | 0.49                        |                     | 0.23                   |

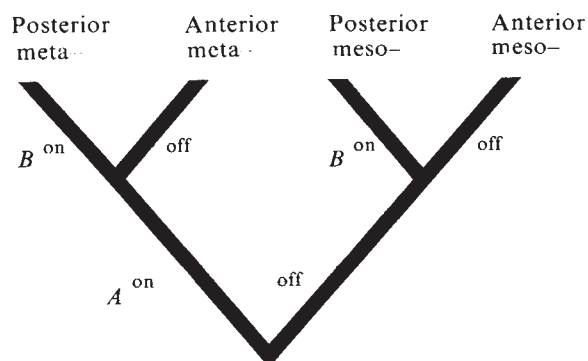
compartment implies that the regeneration blastema is sometimes derived from anterior cells in the original leg. Schubiger<sup>4</sup> showed that anterior fragments<sup>2</sup> can regenerate an entire leg, and Haynie and Bryant<sup>5</sup> have confirmed that regeneration across compartment boundaries can occur. In bithorax homozygotes, anterior cells normally develop as mesothorax. Our results suggest the transformed state may be maintained when these cells form the posterior compartment of the duplicate.

Classification of DFPs by size of deficiency provides no support for the idea that regeneration of a metathoracic posterior compartment depends similarly on recruitment of posterior cells from the original leg. Although we have no direct evidence we prefer the alternative hypothesis which assumes that anterior cells have some finite chance of changing state when they enter the regeneration blastema. The work of Adler (personal communication) provides direct support for this interpretation. Anterior fragments of *bx<sup>3</sup>* haltere disks were occasionally able to form posterior haltere when stimulated to regenerate across the compartment boundary.

Garcia-Bellido<sup>1</sup> argues that compartmentalisation events represent determinative decisions. A determined state is thought of as being specified combinatorially by a sequence of such decisions. A heritable record of each decision is assumed to be maintained by the state (on/off) of a specific 'selector' gene (A, B, in Fig. 2). To accord with the results described above, certain of these states must be reversible in regeneration. It is nevertheless possible that compartmental restrictions are re-established in the regeneration blastema; indeed this is a necessary corollary if compartmentalisation events are required for the determination of cellular fates.

*bx<sup>+</sup>* and *pbx<sup>+</sup>* functions are required until late in development for the differentiation respectively of anterior and posterior metathorax. In normal development these loci must be activated by positional signals specific to the anterior and posterior of the third thoracic segment. Since the mutants act within compartments and behave autonomously in clones, they are believed to be defective in the execution of compartment-specific functions, rather than in the establishment of the compartments<sup>10</sup>. When

**Fig. 2** Binary switch model for determination of compartments of meso- and metathorax after Garcia-Bellido<sup>1</sup>. A and B are hypothetical 'selector' genes.



$bx^+ pbx^+$  third legs duplicate, the posterior compartment is always metathoracic. In the context of the model this implies activation of  $pbx^+$  and thus, heritability of the decision that distinguishes meso- from metathorax. Transformation of the duplicate posterior compartment when the genotype is  $bx^- pbx^+$  therefore implies that  $bx^+$  function is necessary for heritability of this decision. If a memory of the determinative event existed in the state of an independent selector gene (A in Fig. 2), we would not expect a mutation at bithorax to influence activation of postbithorax. Supported by NRC (Canada) grant number A6485 to M.A.R.

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## Application of magnetic microspheres in labelling and separation of cells

POLYMERIC microspheres conjugated to antibodies and lectins have been used previously as cell surface markers for the detection and localisation of antigens and lectin receptors using scanning electron microscopy (SEM) (refs 1–4). In this report we describe a new, more versatile cell surface probe consisting of iron-containing polymeric microspheres tagged with fluorescent dyes and chemically coupled to antibodies or lectins. The magnetic and fluorescent properties of these microspheres have been utilised in the magnetic separation of red blood cells (RBC) and lymphoid cells and in the detection of immunoglobulin (Ig) receptors and wheat germ agglutinin (WGA) receptors on lymphocytes and Hela cells by SEM and fluorescent microscopy.

Magnetic, polymeric microspheres were synthesised from iron oxide ( $Fe_3O_4$ ) particles (Ferrofluidics, Burlington, Massachusetts, No. A01, 5%, w/v) and from methyl methacrylate (MMA), hydroxethyl methacrylate (HEMA), methacrylic acid (MAA), and ethylene glycol dimethacrylate (EGDA) purified as described previously<sup>1,2</sup>. Briefly, a mixture of 1.6 g MMA, 0.9 g HEMA, 0.3 g MAA, 0.2 g EGDA, 1 g iron oxide suspension and 1 g Triton X-405 was de-aerated and polymerised by  $Co \gamma$  irradiation (0.2 Mrad at 0 °C for 60 min)<sup>3</sup>. The Fe microsphere suspension was purified on a mixed-bed ion-exchange column<sup>1</sup> and centrifuged at 30,000g for 45 min at 4 °C on a discontinuous gradient consisting of 20% (w/w) sucrose in the upper layer and 60% (w/w) in the lower layer. Three fractions (brown in colour) were recovered: an upper band in the 20% sucrose fraction (Fe content 6.2%); a lower band in the 20% sucrose layer (Fe content 28.4%); and a pellet (Fe content 42.4%). The upper and lower fractions were used in cell labelling studies. The average size of these particles was 40 nm as measured by SEM.

Copolymer Fe microspheres were derivatised with diaminoheptane by the cyanogen bromide method<sup>1,6</sup> and tagged with 1 mg fluorescein isothiocyanate (FITC) per ml suspension in carbonate buffer, pH 9.5, for 12 h at 25 °C. After removal of excess FITC, the spheres were re-derivatised with diaminoheptane using the carbodiimide method<sup>1</sup>.

Affinity purified antibodies or lectins were coupled to the fluorescent Fe microspheres by the two-step glutaraldehyde reaction<sup>1,7</sup>. A protein concentration of 0.5–1.0 mg ml<sup>-1</sup> and a Fe microsphere concentration of 1 mg ml<sup>-1</sup> was used in the coupling stage. The reaction was carried out for 5–12 h at 25 °C. Unbound protein was separated from the protein-microsphere conjugate either by centrifugation on a discontinuous sucrose gradient<sup>1</sup> or by chromatography on Sepharose 6B. The conjugates could be concentrated with a magnet placed against the wall of a glass vial.

The application of magnetic immunomicrospheres in the separation of cells was tested in two independent systems. In the first, glutaraldehyde-fixed mouse thymocytes indirectly labelled for surface antigens with fluorescein-Fe microsphere-antibody conjugates were mixed in varying proportions with unlabelled human RBC. The mixture was layered over phosphate-buffered saline (PBS) containing 5% bovine serum albumin (BSA) and placed in a magnetic field (horseshoe magnet, 12 lb pull). After 2 h the solution was eluted to separate cells attracted to the magnet from those that were not. Differences in cell morphology and fluorescent labelling were used to analyse the cell fractions. The rate of deflection of labelled cells towards the magnet depended on the extent of labelling and the Fe content of the microspheres. As indicated in Table 1, over 99% of the labelled thymocytes were attracted by the magnet.

In a more applicable system, this technique was used to separate out lymphocytes displaying Ig receptors (B cells) from a mixed population of spleen lymphocytes. Fluorescent Fe microspheres coupled to goat anti-mouse Ig were used to directly label unfixed lymphocytes. Labelled cells could be readily distinguished from unlabelled cells under a fluorescent microscope (Fig. 1). In agreement with literature values<sup>8,9</sup> approximately 37% of the cells were labelled (Table 1). The mixture of labelled and unlabelled cells was subjected to a magnetic field. Previous removal of unbound Fe microspheres was not necessary. After 2 h at 4 °C, 97–99% of the labelled

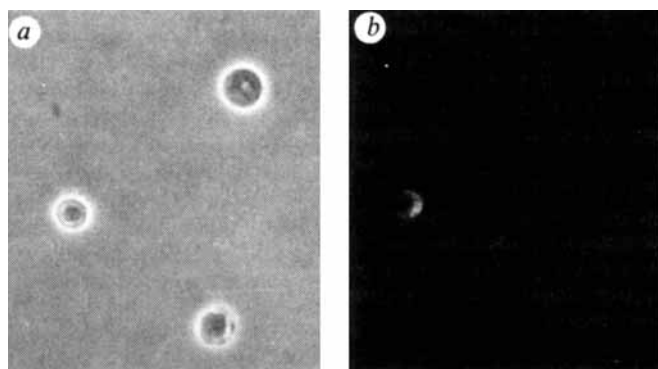


Fig. 1 Mouse spleen lymphocytes directly labelled for surface immunoglobulin (Ig) receptors with fluorescein-Fe microsphere-goat anti-mouse Ig antibody conjugates. *a*, Lymphocytes viewed by phase contrast light microscopy. *b*, The same lymphocytes viewed by fluorescent microscopy. Only one lymphocyte (B cell) is labelled with the conjugates.

cells (principally B lymphocytes) were retained by the magnet, leaving a highly purified population of unlabelled lymphocytes (T cells). Some unlabelled cells, however, were also in the cell population which was retained by the magnet. This resulted largely from nonspecific aggregation of the lymphocytes and nonspecific adhesion of cells to the glass wall of the pipette. Further removal of unlabelled cells from the predominantly labelled cell population could be achieved by a second passage of dissociated cells through the magnetic field. A detailed study of optimal conditions for cell separation will be given elsewhere.

Application of these fluorescent Fe-microspheres as visual markers for SEM was also demonstrated. Figure 2*a* illustrates the labelling of wheat germ agglutinin receptors on cultured Hela cells. Specificity was verified by the absence of binding of the



Table 1 Magnetic cell separation

| Experiment            | Before separation          |                              | % Labelled (F1) | Magnet        | After separation           |                | % Labelled (F1) |
|-----------------------|----------------------------|------------------------------|-----------------|---------------|----------------------------|----------------|-----------------|
|                       | No. of cells counted Total | cells counted Labelled (F1)* |                 |               | No. of cells counted Total | Labelled (F1)* |                 |
| 1<br>(RBC/thymocytes) | 521                        | 429                          | 82.3            | Attracted     | 509                        | 499            | 98.0            |
| 2<br>RBC/thymocytes   | 580                        | 179                          | 30.9            | Not attracted | 500                        | 2              | 0.4             |
| 3<br>(lymphocytes)    | 252                        | 92                           | 36.5            | Attracted     | 532                        | 487            | 91.5            |
| 4<br>(lymphocytes)    | 210                        | 82                           | 39.1            | Not attracted | 520                        | 1              | 0.2             |
|                       |                            |                              |                 | Attracted     | 206                        | 168            | 81.6            |
|                       |                            |                              |                 | Not attracted | 211                        | 3              | 1.4             |
|                       |                            |                              |                 | Attracted     | 144                        | 110            | 76.4            |
|                       |                            |                              |                 | Not attracted | 303                        | 9              | 3.0             |

\*Labelled with fluorescein-Fe microsphere-antibody conjugates.

In RBC-thymocyte experiment 1,  $2.1 \times 10^6$  glutaraldehyde-fixed RBC were mixed with  $8.2 \times 10^6$  fixed mouse thymocytes labelled sequentially with rabbit anti-thymocyte antiserum followed by fluorescein-Fe microsphere-goat anti-rabbit immunoglobulin conjugates. Experiment 2 was carried out in similar conditions, except that  $8.1 \times 10^6$  RBC were mixed with  $3.3 \times 10^6$  thymocytes. The number of fluorescent cells was measured. Approximately,  $5 \times 10^6$  mixed cells were layered on a 5% BSA-PBS solution. A magnet (12 lb pull) was placed against the wall of a 0.9-cm diameter column at the interface. After 2 h at 4 °C the column was gently eluted with PBS to separate cells attracted to the magnet from those which were not. The magnet was then removed from the side of the column, and the cells pulled to the side of the column were displaced by shaking the column. The two cell fractions were analysed for fluorescent (F1) labelling and for cell type using a Leitz Dialux fluorescent microscope. In experiments 3 and 4,  $1 \times 10^6$  mouse spleen lymphocytes purified by the Ficoll isopaque method<sup>13</sup> were directly labelled with 0.1 ml fluorescein-Fe microsphere-goat antimouse Ig conjugates at 4 °C. Cells were washed and the percentage of cells with fluorescein label was measured. Approximately  $5 \times 10^6$  cells were then layered on a Ficoll isopaque layer and subjected to magnetic separation as described above.

WGA-microsphere conjugate in the presence of *N*-acetylchitobiose, an inhibitor of WGA (Fig. 2b).

These new cell surface reagents have potential applications in biochemical and microscopic studies of specific components on cell surface membranes. The magnetic properties of these spheres can be utilised to isolate specific types of cells. The magnetic cell sorting technique described here is quite simple, but more sophisticated instrumentation can be envisioned to continuously separate cells which differ in the number, as well as the nature of surface molecules. Such techniques would be particularly useful for processing large numbers of cells for biochemical and immunological studies. Magnetic properties can also be used in the separation of labelled cell surface membranes from intracellular membranes, as well as in the purification of specific membrane-bound receptors which have been solubilised in mild detergents. Stronger magnetic fields may be required in such applications. Alternatively, the dense properties of these spheres can be exploited to separate membranes and receptors by density or velocity perturbation techniques using ultracentrifugation<sup>10,11</sup>.

The iron content of these microspheres also permits their use as visual markers for transmission electron microscopy (TEM). This enables one to correlate labelling information derived from light and SEM with cellular ultrastructure obtained by TEM. In principle, similar reactions used in the synthesis of these iron-

containing polymeric spheres can be adapted for preparing microspheres containing gold or other heavy metals which have advantages for SEM and TEM (refs 4, 12).

Finally, these or related reagents may have future clinical applications. Magnetic microspheres carrying cytotoxic drugs, enzymes or radioisotopes could, in principle, be localised in a certain tissue of a body such as a tumour, by applying powerful magnets and serve as diagnostic or therapeutic agents.

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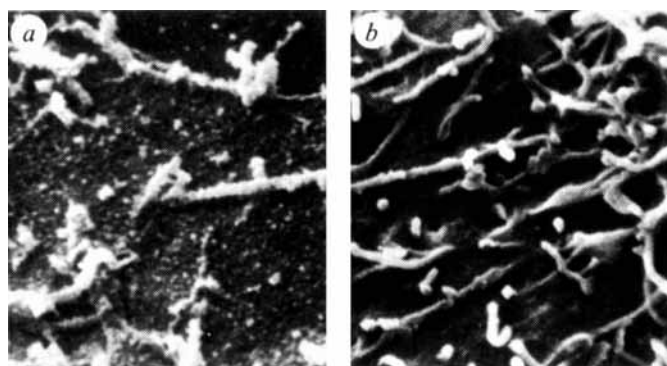


Fig. 2 Scanning electron micrographs of the surface of a cultured Hela cell labelled with fluorescein-Fe microsphere-WGA conjugates in a, the absence and b, the presence of the WGA inhibitor, *N*-acetylchitobiose. Microspheres bind only when the inhibitor is absent during labelling.

## Formation and resealing of pores of controlled sizes in human erythrocyte membrane

APPLICATION of an electric pulse, at field intensities of a few kV cm<sup>-1</sup> and of duration in the  $\mu$ s range, to an isotonic suspension of erythrocytes is known to cause haemolysis of the red

cells<sup>1-4</sup>. Studies from different laboratories suggest that the haemolysis is due to the field-induced transmembrane potential<sup>1,3,4</sup>. Our recent experiments<sup>5</sup> indicate that once the transmembrane potential reaches a threshold of approximately 1 V, which corresponds to an applied field of  $2.2 \text{ kV cm}^{-1}$ , the erythrocyte membrane becomes leaky to normally impermeant ions or molecules. The permeation of solutes leads to the swelling and eventual lysis of the red cells. This type of haemolysis is known as colloid osmotic haemolysis<sup>6,7</sup>. The voltage-induced permeability change is consistent with the formation of pores in the membrane. We show here that the size of these pores can be varied in a controlled manner, and that the leaky membrane can be resealed while the haemolysis is prevented. Foreign molecules have successfully been incorporated into the resealed, but otherwise intact, erythrocytes.

In the experiment shown in Fig. 1, erythrocytes in an isotonic NaCl solution were treated with a  $3.7 \text{ kV cm}^{-1}$ , 20- $\mu\text{s}$  pulse, and the subsequent change in cell volume  $V$  was monitored by light scattering measurement. In these conditions, intracellular  $\text{K}^+$  leaks out, being replaced with  $\text{Na}^+$ , within a few

of sucrose solution reduced the external NaCl concentration further, and the cells began to shrink due to the efflux of NaCl (curve *a*).

When the added substance was xylitol (MW 152 whereas sucrose is 342), only partial blocking of the cell swelling was achieved as shown in curve *c*. The volume change in this situation is expected to satisfy the following relation

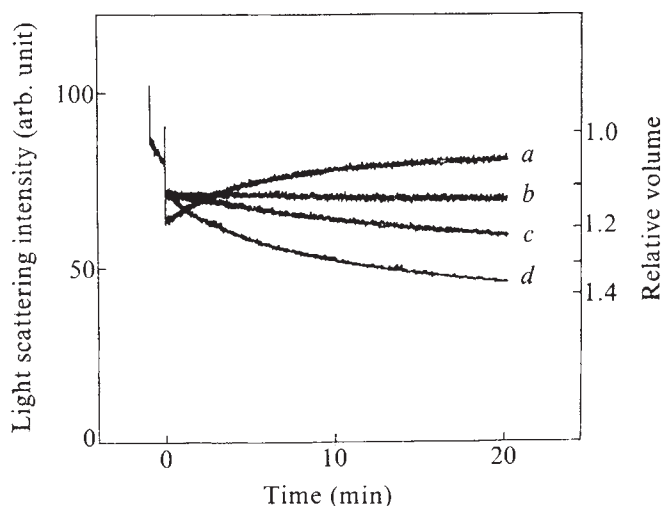
$$dV/dt = (j_x + j_{\text{NaCl}})V/C \quad (1)$$

where  $j_x$  and  $j_{\text{NaCl}}$  are the net influxes of xylitol and NaCl respectively, in  $\text{mOsmol l}^{-1} \text{ cells h}^{-1}$  and  $C$  the total osmotic concentration of the medium in  $\text{mOsmol l}^{-1}$ . At  $t \approx 0$  or  $V \approx V_0$ , the net influx  $j_{\text{NaCl}}$  is negligible, as shown in curve *b*, and the swelling is almost entirely due to the xylitol influx  $j_x$ . Thus the rate of permeation of xylitol molecule  $k_x$  can be roughly estimated as

$$k_x = j_x/C_x = (V^{-1}dV/dt)_{t=0}(C/C_x) \quad (2)$$

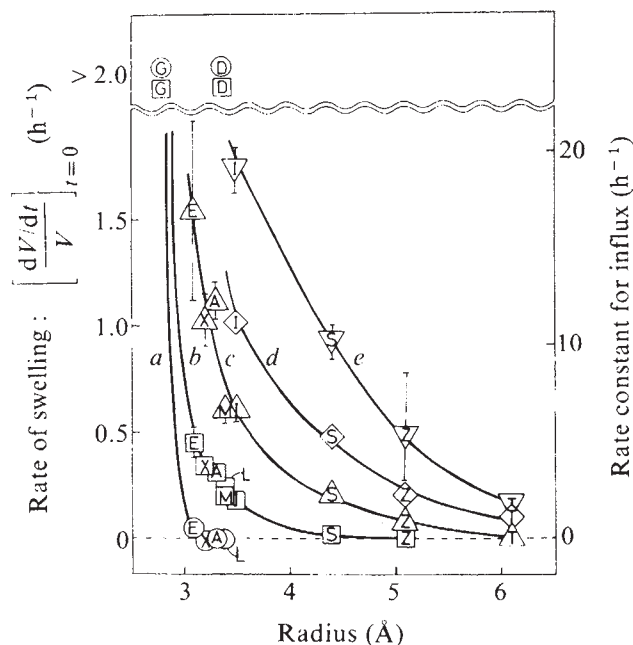
where  $C_x$  is the external osmotic concentration of xylitol ( $C/C_x = 11$ ).

In this way, the permeability of the pulse-treated membrane to 11 different carbohydrates was measured in various conditions. (Tracer influx measurements on a few selected carbohydrates gave values consistent with those obtained by the above method.) The results are plotted in Fig. 2 against the average radius of the tested molecule. Curve *a* gives data for the untreated cells; molecules larger than erythritol do not enter the cells to any appreciable amount, except D-glucose which is known to be carried by a specific transport system<sup>7</sup>. This agrees with the idea that pores of a radius of about 3.5–4.2 Å exist in human erythrocyte membrane<sup>8</sup>. Curve *b* shows



**Fig. 1** Changes in cell volume after the electric pulsation. Washed human erythrocytes were suspended in an isotonic NaCl solution (150 mM NaCl, 7 mM phosphate buffer, pH 7.0) at a volume concentration of 1%, and treated with a single square-wave electric pulse of intensity  $3.7 \text{ kV cm}^{-1}$ , duration 20  $\mu\text{s}$ , between a pair of stainless steel electrodes. After 10–20 s, an aliquot was taken and mixed with 50 volumes of the isotonic NaCl solution, and the change in light scattering intensity at 600 nm was recorded with an Aminco-Bowman spectrofluorometer equipped with a magnetic stirrer. The scattering intensity was calibrated against the cell volume  $V$  by a separate haematocrit measurement, as shown in the right-hand scale which applies to the  $t > 0$  portion of curves *b–d*. When  $V$  reached 1.1 times that of the untreated  $V_0$ , the following additions were made ( $t = 0$ ). Curve *a*, 2/10 volume of isotonic (272 mM) sucrose solution to 1 volume of the suspension; curve *b*, 1/10 volume of the isotonic sucrose solution; curve *c*, 1/10 volume of isotonic (285 mM) xylitol solution; curve *d*, 1/10 volume of the isotonic NaCl solution. Temperature was  $25^\circ\text{C}$ . In these conditions, the extent of haemolysis is negligible for the 20-min period shown; however, all the cells haemolyse by 15 h unless the carbohydrates are added.

minutes. Further entry of NaCl causes the swelling of the red cells<sup>5</sup>. When  $V$  reached  $1.1 V_0$  (where  $V_0$  is the volume of the untreated cells) a small amount of isotonic solution of various substances was added to the suspension (time  $t = 0$ ). Curve *d* in Fig. 1 is a control, where 1/10 volume of isotonic NaCl was added. The cells kept swelling because of the continuous influx of NaCl. However, when the same amount of sucrose solution was added, the swelling immediately stopped as shown in curve *b*. A separate tracer experiment showed that the cell membrane is practically impermeable to sucrose in these conditions. Therefore, the above result indicates that the reduction of the external NaCl concentration by 9% is just sufficient to abolish the electrochemical gradient, or the resultant uptake, of NaCl. As expected, the addition of a larger amount



**Fig. 2** Permeability of the pulse-treated erythrocyte membrane to various carbohydrate molecules. Curve *a*, untreated cells; curve *b*, cells treated with a  $3.7 \text{ kV cm}^{-1}$ , 20- $\mu\text{s}$  pulse in isotonic NaCl solution; curve *c*,  $5.3 \text{ kV cm}^{-1}$ , 20- $\mu\text{s}$ , in NaCl; curve *d*,  $3.7 \text{ kV cm}^{-1}$ , 80  $\mu\text{s}$ , in NaCl; curve *e*,  $3.7 \text{ kV cm}^{-1}$ , 20  $\mu\text{s}$ , in a 3:7 mixture of isotonic NaCl and isotonic sucrose solutions. After the pulsation, swelling of the cells due to the influx of various carbohydrate molecules was recorded as in curve *c* of Fig. 1. The rate of swelling was determined from the initial slope of the swelling curve; the rate of permeation was calculated from equation (2). The average radius in the abscissa denotes  $(r_1 r_2 r_3)^{1/3}$  where the  $r_i$  values are three orthogonal radii measured in space-filling molecular models. G, glycerol; E, meso-erythritol; X, xylitol; A, D-arabitol; D, D-glucose; L, L-glucose; M, D-mannitol; I, myo-inositol; S, sucrose; Z, melezitose; T, stachyose.



Table 1 Incorporation of sucrose in resealed erythrocytes

| Treatment                                                | Procedure                                                                                                                  | Medium | Time      | % survival | Assay<br>Relative<br>cell volume | Sucrose<br>content | Na <sup>+</sup><br>content<br>(mmol l <sup>-1</sup> cells) | K <sup>+</sup><br>content |
|----------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|--------|-----------|------------|----------------------------------|--------------------|------------------------------------------------------------|---------------------------|
| a Pulsation<br>3.7 kV cm <sup>-1</sup> ,<br>80 μs, 25 °C | NaCl 135 mM, NaP* 6 mM, sucrose 27 mM,<br>pH 7.0                                                                           |        | before a  | 100        | 1.00                             | 0.0                | 91                                                         | 10                        |
| b Resealing 1<br>37 °C, 1 h                              | NaCl 119 mM, NaP 7 mM, sucrose 27 mM,<br>stachyose 21 mM, MgSO <sub>4</sub> 2 mM, pH 7.2                                   |        | after b   | 99         | 0.99                             | 3.2                | 92                                                         | 11                        |
| c Resealing 2<br>37 °C, 6 h                              | NaCl 107 mM, KCl 5 mM, sucrose 27 mM,<br>stachyose 21 mM, adenosine 3 mM,<br>inosine 1 mM, supplement†, pH 7.5             |        | after c   | 97         | 0.94                             | 3.5                | 98                                                         | 17                        |
| d Resealing 3<br>37 °C, 12 h                             | NaCl 107 mM, KCl 5 mM, CaCl <sub>2</sub> 2 mM,<br>stachyose 40 mM, adenosine 0.3 mM,<br>inosine 0.1 mM, supplement, pH 7.5 |        | after d   | 95         | 1.01                             | 3.2                | 81                                                         | 27                        |
| e Test incubation<br>37 °C, 72 h                         | NaCl 133 mM, KCl 5 mM, CaCl <sub>2</sub> 2 mM,<br>adenosine 0.15 mM, inosine 0.05 mM,<br>supplement, pH 7.5                |        | 24 h in e | 74         | 1.00                             | 3.3                | 63                                                         | 42                        |
|                                                          |                                                                                                                            |        | 48 h in e | 65         | 0.99                             | 3.3                | 55                                                         | 46                        |
|                                                          |                                                                                                                            |        | 72 h in e | 58         | 0.95                             | 3.2                | —                                                          | —                         |

\* NaP, Sodium phosphate buffer.

† Supplement: NaP 7 mM, MgSO<sub>4</sub> 2 mM, D-glucose 11 mM, chloramphenicol 0.1 mg ml<sup>-1</sup>, penicillin G 500 units ml<sup>-1</sup>, bovine serum albumin 30 mg ml<sup>-1</sup>. Sucrose contained trace amount of [<sup>14</sup>C] sucrose.

Volume concentration of the cells was ~20% at stage a, 10% at b, 3–5% at c through e. The suspension was gently rocked during the incubations. In e, medium was changed at every 12 h, during which pH dropped by 0.2–0.3 unit. Percentage survival was calculated from the amount of haemoglobin released in the supernatant. Relative cell volume was assumed to be inversely proportional to the amount of haemoglobin contained in a unit volume of packed cells (haemoglobin content of the untreated cells was 315 ± 6 g l<sup>-1</sup> cells). Sucrose content was determined from the radioactivity of the cells packed in microhaematocrit tubes. Na<sup>+</sup> and K<sup>+</sup> were assayed by flame photometry. Assay values except % survival refer to survived (unlysed) cells. All values are averages of several determinations on two independent samples; variations were within ±1% for % survival, ±0.02 for cell volume, ±0.2 mmol l<sup>-1</sup> cells for sucrose, ±5 mEq l<sup>-1</sup> cells for Na<sup>+</sup> and K<sup>+</sup>.

that the treatment with a 3.7 kV cm<sup>-1</sup>, 20, μs pulse increases the critical size for permeation: molecules smaller than sucrose can penetrate the membrane, and the rate decreases with the size of the molecule. Again, an exception to this curve is D-glucose; the specific transport system seems to be intact even after the pulse treatment. As can be seen from curves c, d and e, larger pores are obtained either by using a higher field intensity, by increasing the pulse duration, or by reducing the ionic strength of the pulsation medium.

As suggested by curve b in Fig. 1, addition of a sufficient

amount of impermeant substance to the suspension of pulse-treated erythrocytes retards the haemolysis indefinitely. While the cells are prevented from lysis, the membrane spontaneously reseals as in the case of ghosts obtained by hypotonic lysis<sup>9</sup>. The resealing process is strongly temperature-dependent, as shown in Fig. 3. The ordinate is the rate of swelling measured in isotonic NaCl; as before, this rate is proportional to the net influx of NaCl. At 37 °C the treated membrane rapidly regains its impermeability to cations, whereas at 3 °C the cells remain highly permeable even after 20 h.

The electric pulsation followed by an appropriate resealing procedure makes it possible to prepare erythrocytes (not ghosts) with altered intracellular compositions. For example, the ionic composition of the resealed cells reflects that of the resealing medium: incubation in NaCl or KCl media yields cells loaded predominantly with Na<sup>+</sup> or K<sup>+</sup> respectively. Although the alteration of cellular cations can also be achieved by lactose treatment<sup>10</sup> or chemical modification of the cell membrane<sup>11</sup>, the present method allows the incorporation of larger molecules such as sucrose by introducing pores of adequate size. Results of a typical experiment are shown in Table 1. The intactness of the loaded cells was tested by incubation for an additional 72 h in a simulated physiological medium at 37 °C. Although some haemolysis occurred, especially during the first 24 h of the test incubation, about 60% of the original cells survived the 3-d period. The cells in the medium assumed either normal biconcave or slightly cup-shaped disk forms and maintained approximately normal cell volume. The resealed erythrocytes accumulated K<sup>+</sup> and extruded Na<sup>+</sup> against a concentration gradient, indicating that the pores were almost completely annealed and the Na–K pump was intact.

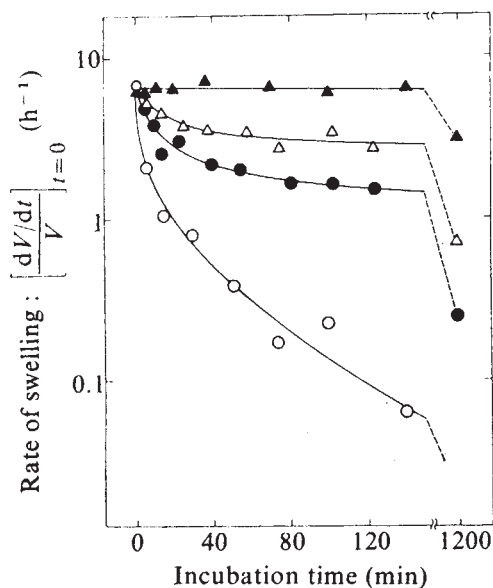
At least two applications of the present technique are conceivable: (1) the alteration of intracellular compositions will simplify experimental designs, especially in transport studies. (2) Erythrocytes may be used as intravenous drug reservoirs; gradual release from loaded erythrocytes could help maintain the drug level in a patient's circulation.

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Fig. 3 Time courses of the resealing of pulse-treated erythrocytes at different temperatures. ▲, 3 °C; △, 17 °C; ●, 25 °C; ○, 37 °C. The washed erythrocytes in isotonic NaCl were treated with a 3.7 kV cm<sup>-1</sup>, 80-μs pulse at 25 °C, and immediately mixed with a large volume of 85:15 mixture of isotonic NaCl and isotonic stachyose solutions kept at the specified temperatures. At intervals, an aliquot was taken and added to an isotonic NaCl solution of the same temperature, and the initial rate of swelling was measured as described in Fig. 1. Haemolysis in the incubation media (NaCl–stachyose) was negligible except at 3 °C, where a small portion of the cells haemolysed by 20 h presumably because of the very slow penetration of stachyose.



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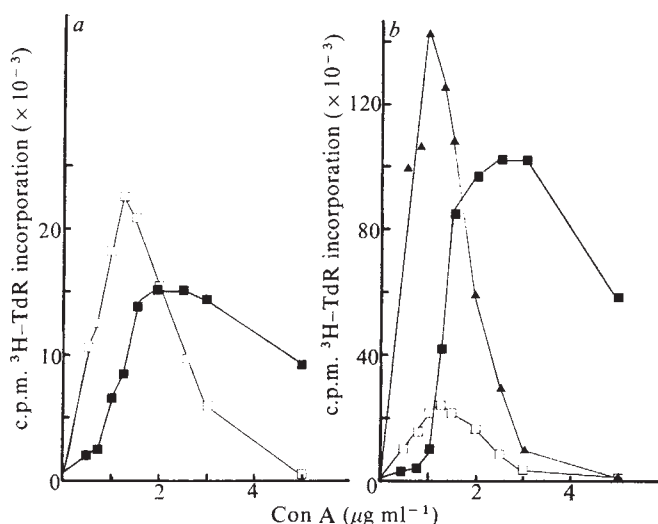
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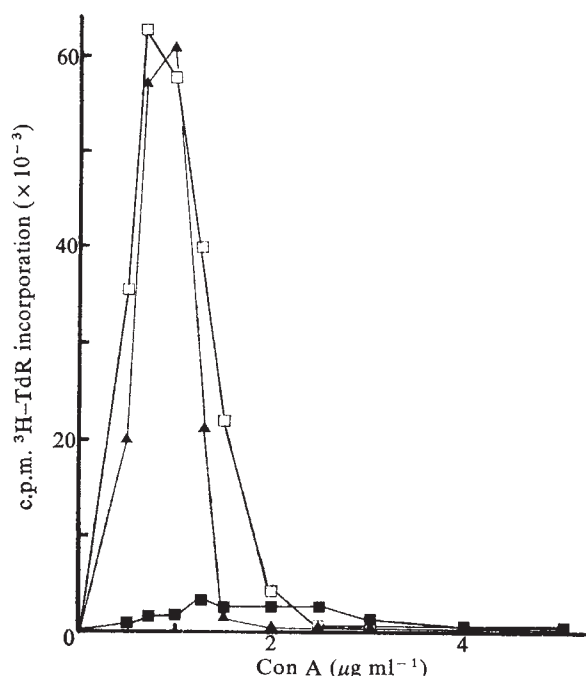
## Activation of mouse spleen cells by a single short pulse of mitogen

THE stimulation of lymphocytes by polyclonal mitogens such as concanavalin A (con A) is a useful system for analysing the activation of cells out of the resting state,  $G_0$ , into the division cycle. Optimal stimulation of the lymphocytes with con A, however, requires presence of the mitogen for about 20 h (ref. 1), and the cells transfer from  $G_0$  into  $G_1$  in an unsynchronised manner<sup>2</sup>. This lack of synchrony makes biochemical analysis of activation complicated because sequential events of the cell cycle become superimposed. I show here that a single short pulse of con A will irreversibly commit a significant proportion of murine spleen cells into the division cycle. This relatively synchronised stimulation of cells enables the requirements for activation to be analysed separately from subsequent events as the cells progress through the cell cycle. For example, it has been shown that the presence of colchicine during a 2-h exposure to con A inhibits mitogenic activation.

Spleen cells from 6–10-week-old male BALB/c mice were cultured in the absence of serum (Fig. 1 legend), and mitogenesis was measured by the incorporation of  $^3\text{H}$ -thymidine



**Fig. 1** Dose-response curves of BALB/c spleen cells to con A after 3-h, 28-h and 52-h exposure to the lectin. Labelling for *a*, 23–28 h; *b*, 47–52 h. Washed spleen cells from a single mouse were diluted to  $5 \times 10^6 \text{ ml}^{-1}$  cells with growth medium (RPMI 1640 plus 100 U  $\text{ml}^{-1}$  penicillin and 100  $\mu\text{g ml}^{-1}$  streptomycin) containing the required concentration of con A. Aliquots (100  $\mu\text{l}$ ) of these cells were dispensed into flat-bottomed wells of a microtitre tray and cultured in a humidified atmosphere of 10%  $\text{CO}_2$ , 7%  $\text{O}_2$  and 83%  $\text{N}_2$  at 37 °C. Con A was removed by complete replacement of the supernatant with prewarmed growth medium containing the appropriate concentration of  $\alpha$ -MM (see text). DNA synthesis was measured using standard techniques after labelling each culture with 0.5  $\mu\text{Ci}$   $6\text{-}^3\text{H}$ -thymidine (specific activity = 2.0 Ci  $\text{mmol}^{-1}$ ) for 5 h. Each point represents the mean of 5 replicate cultures. ■, Con A 0–3 h; □, con A 0–28 h; ▲, con A 0–52 h. The same results were obtained in three separate experiments.



**Fig. 2** Dose-response curves of BALB/c thymocytes to con A after 3 h, 28 h and 52 h exposure to the lectin. Experimental details as in the legend to Fig. 1. DNA synthesis was measured between 47–52 h. ■, Con A 0–3 h; □, con A 0–28 h; ▲, con A 0–52 h.

into cellular DNA and the stimulation index (SI) calculated as c.p.m. con A-treated cultures/c.p.m. control cultures. Con A ( $1 \mu\text{g ml}^{-1}$ ) was reduced to non-mitogenic levels after 3 h by changing the growth medium to remove free con A and adding 5 mM  $\alpha$ -methyl-D-mannoside ( $\alpha$ -MM) to displace cell-bound con A; this ratio of sugar to lectin (that is, 5 mM:  $1 \mu\text{g ml}^{-1}$ ) was used experimentally. The concentration of con A required for maximal mitogenic stimulation was dependent on the duration of exposure to the lectin (Fig. 1). Cells continuously exposed to con A gave a very sharp dose-response curve at 52 h which peaked at  $1 \mu\text{g ml}^{-1}$  with SI = 252, and fell to background levels of DNA synthesis above  $3 \mu\text{g ml}^{-1}$  con A. In contrast, identical cells given a 3-h pulse of con A showed a broad response curve peaking at  $2\text{--}3 \mu\text{g ml}^{-1}$  at both 28 h (SI = 25) and 52 h (SI = 183). This change in the dose-response curve is not a result of a change in the optimal dose requirements during the development of the mitogenic response, since the peak position was constant at both 28 h and 52 h following exposure to con A for either 3 h or 24 h (Fig. 1). It is relevant to note that when the dose-response curves were repeated in identical conditions, but using BALB/c thymocytes instead of spleen cells, 3-h exposure to con A did not produce a significant mitogenic response (maximal s.e. = 5.4 at  $1.25 \mu\text{g ml}^{-1}$  con A) although the same cells responded well after prolonged exposure to the lectin (Fig. 2).

This time-dependent shift in the dose-response curve of spleen cells may be caused by a variety of cellular responses to con A which develop at different rates and show different sensitivity to the lectin. For example, concentrations of con A high enough to induce a complete activation signal in a short time may thereafter initiate an inhibitory effect dependent on prolonged exposure of the cells to con A. Lower concentrations of con A, although less effective during the inhibitory phase, require longer to generate a complete activation signal. A similar explanation has recently been suggested by McClain and Edelman<sup>3</sup>. On the other hand, the possibility that  $\alpha$ -MM failed to reduce bound con A to non-mitogenic levels must be considered, although this is unlikely since addition of  $\alpha$ -MM abolished



Table 1

| Experiment no.                                       | Time (h)                                                                                                                                                  | $^3\text{H}$ -TdR incorporation (c.p.m.)                             | $b/a \times 100$ |
|------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------|------------------|
|                                                      | 0 1 2 3                                                                                                                                                   |                                                                      |                  |
| 1 { $\begin{smallmatrix} a \\ b \end{smallmatrix}$ } | $\xleftarrow{\quad} \text{C} \xrightarrow{\quad}$                                                                                                         | $\begin{Bmatrix} 169 \pm 14 \\ 178 \pm 33 \end{Bmatrix}$             | 105%             |
| 2 { $\begin{smallmatrix} a \\ b \end{smallmatrix}$ } | $\xleftarrow{\quad} \text{C} \xrightarrow{\quad} \xleftarrow{\quad} \text{con A} \xrightarrow{\quad} \xleftarrow{\quad} \text{con A} \xrightarrow{\quad}$ | $\begin{Bmatrix} 75,142 \pm 6,927 \\ 67,607 \pm 4,677 \end{Bmatrix}$ | 87.3%            |
| 3 { $\begin{smallmatrix} a \\ b \end{smallmatrix}$ } | $\xleftarrow{\quad} \text{con A} \xrightarrow{\quad} \xleftarrow{\quad} \text{con A} + \text{C} \xrightarrow{\quad}$                                      | $\begin{Bmatrix} 28,182 \pm 1,300 \\ 10,880 \pm 908 \end{Bmatrix}$   | 38.6%            |
| 4 { $\begin{smallmatrix} a \\ b \end{smallmatrix}$ } | $\xleftarrow{\quad} \text{con A} \xrightarrow{\quad} \xleftarrow{\quad} \text{con A} \xrightarrow{\quad} \text{C} \xrightarrow{\quad}$                    | $\begin{Bmatrix} 43,482 \pm 1,727 \\ 40,577 \pm 5,556 \end{Bmatrix}$ | 93%              |
|                                                      | 0 2 24 52                                                                                                                                                 |                                                                      |                  |
| 5 { $\begin{smallmatrix} a \\ b \end{smallmatrix}$ } | $\xleftarrow{\quad} \text{con A} \xrightarrow{\quad} \xleftarrow{\quad} \text{con A} \xrightarrow{\quad} \xleftarrow{\quad} \text{C} \xrightarrow{\quad}$ | $\begin{Bmatrix} 16,863 \pm 724 \\ 4,532 \pm 322 \end{Bmatrix}$      | 26.8%            |

Murine spleen cells were cultured as described in the legend to Fig. 1. Cultures were exposed to  $5 \mu\text{g ml}^{-1}$  con A and  $0.5 \mu\text{M}$  colchicine (C) during the times indicated by the horizontal bars, and these agents were removed by complete replacement of the supernatant with prewarmed growth medium containing 25 mM  $\alpha$ -MM. Controls were always treated identically to experimental cultures to ensure that the effect of medium change and sugar addition did not interfere with the results.  $^3\text{H}$ -thymidine uptake was measured between 47–52 h and each value represents the mean of five cultures. In each experimental group  $a$  were the control cultures.

mitogenesis in control experiments. In addition, the different characteristics of cell populations responding to acute and prolonged exposure to con A (that is, the shape of the dose-response curves and duration of DNA synthesis) show that the  $\alpha$ -MM did not cause a simple shift in the dose-response curve. Further confirmation of the efficacy of  $\alpha$ -MM is apparent in the experiments with thymocytes, which responded well to prolonged exposure to con A but were unable to respond to a 3-h pulse.

These results are pertinent to the analysis of early cellular responses to con A which constitute an activation signal. Such analysis often uses con A concentrations which give maximal DNA synthesis at 48 h in the continuous presence of con A, but it is clear that this concentration of con A is insufficient to generate a significant activation signal within a short time. In contrast, short pulses of high concentrations of con A can activate cells within a few hours (Fig. 1). From these results it is suggested that in order to analyse the early events in the activation of cells from  $G_0$  to  $G_1$  it may be important to use a concentration of con A high enough to generate an activation signal within a short period.

The finding that lymphocytes will transform after a single short pulse of con A contrasts with results of Toyoshima *et al.*<sup>4</sup>, who found that two 3-h pulses, separated by a gap of 12 h, are necessary for optimal con A stimulation ( $\text{SI} = 3.9$ ) and that a single 3-h pulse did not induce DNA synthesis ( $\text{SI} = 1.2$ ) in murine spleen cells. The discrepancy between this result and my own may be due partly to the presence of serum in the experiments of Toyoshima *et al.*, since their control cultures showed a high background of DNA synthesis presumably caused by the nonspecific mitogenic action of serum. In my cultures the absence of serum maintains the cells in a quiescent state but able to respond specifically to added mitogen, as indicated by the high stimulation indices of around 200.

Colchicine is known to bind tubulin and thereby reduce the microtubule system in cells. Its effect on lymphocyte mitogenesis in the continuous presence of con A has led to conflicting published results. For example, Betel and Martijnse<sup>5</sup> found that colchicine had no effect on the activation of rat lymphocytes by con A, but that the drug did block subsequent events in the cell cycle. In contrast, others<sup>6</sup> found that the inhibitory effect of colchicine was limited to the time of lymphocyte activation. In my own experiments using synchronously activated spleen cells, I found that short pulses of colchicine during the activation time caused a marked inhibition of mitogenesis (see Table

1). A 1-h pulse of colchicine immediately following con A treatment, however, had little effect on con A mitogenesis (Table 1). This supports the idea that colchicine inhibits events which are involved in the activation of cells. It was also found that the presence of colchicine during the final 24 h of a 52-h culture of cells (activated by a 2-h pulse of con A) caused marked inhibition of DNA synthesis measured at 47–52 h. These results suggest that an intact microtubular system is necessary for both mitogenic activation and the normal progression of the cell through the cell cycle.

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## Development of mast cells from grafted bone marrow cells in irradiated mice

BOTH basophilic granulocytes and tissue mast cells are known to have receptors for immunoglobulin E (IgE) and granules which contain histamine and heparin<sup>1,2</sup>. In spite of their similar physiological role, these two types of cells are thought to be independent cell lineages; the former are derived from the bone marrow, and the latter are connective tissue cells<sup>2</sup>. The differentiation of mast cells from their precursors, which were supposed to be undifferentiated mesenchymal cells, and the proliferation of these precursors were reported to occur in the skin of mice<sup>3,4</sup>. These results do not necessarily exclude the possibility that the precursor cells themselves are originated from the bone marrow, however. To examine such a possibility, we investigated whether mast cells of the donor origin appeared in radiation chimaeras, using the giant granules of beige (Chediak-Higashi syndrome) mouse<sup>5</sup> as a quantitative marker for mast cells. We have found that tissue mast cells can be derived from grafted bone marrow cells in irradiated mice.

Beige (C57BL-Bg/Bg<sup>+</sup>) mice (Jackson Laboratory, Bar Harbor, Maine) and normal (C57BL-+/+) mice were bred in

Table 1 Appearance of donor-type mast cells in various tissues

| Mouse no. | Time after transplantation (d) | Caecum | Mesentery | % of donor-type mast cells<br>Skin | Glandular stomach | Forestomach |
|-----------|--------------------------------|--------|-----------|------------------------------------|-------------------|-------------|
| M12       | 9                              | 0      | 0         | 0                                  | 0                 | 0           |
| M25       | 24                             | 0      | 0         | 0                                  | 0                 | 0           |
| M28       | 42                             | 9      | 0         | 0                                  | 6                 | 0           |
| M04       | 63                             | 96     | 0         | 0                                  | 79                | 1           |
| M01       | 96                             | 78     | 2         | 0                                  | 66                | 5           |
| M10       | 126                            | 79     | 6         | 0                                  | 57                | 26          |
| M26       | 152                            | 98     | 11        | 14                                 | 87                | 46          |
| M54       | 180                            | 94     | 2         | 0                                  | 56                | 36          |

The appearance of mast cells with giant granules was monitored at various times after the transplantation of bone marrow cells of the beige mice into the irradiated normal mice. The method of transplantation was described previously<sup>12</sup>.

our laboratory. At an age of 3 months, normal mice were irradiated (800 rad) and injected intravenously with bone marrow cells ( $5 \times 10^6$ ) of either beige or normal mice. On various days after the cell injection, a blood sample was obtained from a lateral tail vein of recipient mice. The mice were then killed; the caecum, the stomach and a piece of dorsal skin were removed and fixed in 10% buffered formalin (pH 7.2). The stretch preparation of the mesentery was also fixed in 10% formalin. Blood smears were fixed in formaldehyde vapour, stained with Sudan black B, and counterstained with Giemsa solution<sup>6,7</sup>. More than 100 neutrophils with distinctly segmented nuclei were counted. Tissues were embedded in paraffin; sections and the stretched mesentery were stained with acidified toluidine blue<sup>8</sup>. In most cases, more than 200 mast cells were counted in each tissue.

Over 90% of neutrophils had giant granules after the 8th day of the cell injection, and were considered to be of donor origin.

Examination of tissues under the microscope revealed the following. (1) Most of mast cells in the caecum were of donor origin after the 63rd day of the cell injection (Table 1). (2) Most of mast cells in the mesentery and the skin were of host type even on the 180th day (Table 1). (3) In the glandular stomach, mast cells of the donor type were predominant, whereas those of the host type were superior in the forestomach which is covered with stratified squamous epithelium (Table 1). (4) No mast cells with giant granules were found in any of control mice which had received the marrow cells of the normal mice after irradiation.

Our results clearly shown that most of the mast cells under glandular epithelium are originated from the grafted marrow cells and that mast cells of the host remained predominant under squamous epithelium and in the mesentery. As mast cells constitute less than 0.01% of the nucleated cells in the bone marrow of C57BL mice (both Bg<sup>+</sup>/Bg<sup>+</sup> and +/+), unpublished), and as it takes about 40 d for the mast cells of the donor origin to appear, the donor-type mast cells found in various tissues seem to be derived from the precursor cells rather than from the mature mast cells contained in the marrow of the donor.

Two possibilities are postulated to explain the difference in the proportion of the donor-type mast cells in the various tissues. (1) Mast cells under glandular epithelium may be different from those of other tissues. Enerbäck found that granules of some mast cells in the intestinal canal of the rat were not fixed with 10% formalin<sup>8</sup>. Moreover, intestinal mast cells stained according to the method of Enerbäck decreased in nude mice<sup>9</sup>, although mast cells of the skin increased in these mice<sup>10,11</sup>. As 10% formalin was used for fixation in the present experiment, the mast cells counted in the intestinal canal should be different from the "mucosal mast cells" of Enerbäck. (2) Mast cells in the various tissues are of the same kind, but the turnover rate of the cells is different in each tissue presumably because the differentiation from the precursor cells is locally controlled. Further investigation is in progress to see whether the cellular contact or chemical factor, which act only in the short range, have a major role for the differentiation of mast cells.

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## Evidence for Fc receptors on rabbit yolk sac endoderm

APPLICATION of the erythrocyte-antibody rosette technique (EA rosette) to cell cultures of mouse yolk sac membranes has provided evidence of Fc receptors on yolk sac endoderm of this species<sup>1</sup>. Binding of immunoglobulin (Ig) to Fc receptors forms an integral part of a hypothesis proposed by Brambell<sup>2</sup> to account for selective materno-foetal transmission of antibodies. In this hypothesis the receptor protects attached Ig when a transporting endocytotic vesicle (be it in syncytiotrophoblast, yolk sac endoderm, or gut enterocytes, according to the species) contacts proteolytic enzymes after fusion with lysosomes. Little is known about selective transport of antibodies across mouse yolk sac endoderm and it is not clear to what extent these results show binding specific for transport of Ig across the endodermal cells, or merely reflect some special mechanism for endocytosing this particular class of proteins. Studies of rabbit yolk sac endoderm have not shown whether specific binding of Ig takes place; thus bovine IgG, which is poorly transported across rabbit yolk sac endoderm<sup>3,4</sup>, although readily endocytosed<sup>5</sup>, binds to the same extent as rabbit IgG to membrane components isolated from the cells<sup>6</sup>. On the other hand, compared with rabbit IgG, relatively little specific binding of bovine IgG was found when these proteins were incubated *in vitro* with formalin-fixed rabbit yolk sac<sup>7</sup>. We have now applied the EA rosette technique to rabbit yolk sac endoderm and find Fc receptors which bind both rabbit



and human IgG, but not bovine IgG, pointing to their association with selective Ig transport.

To obtain yolk sac endodermal cells, conceptuses at 24–26 d of gestation were removed intact from pregnant Dutch or New Zealand White rabbits and washed free of contaminating blood with Hanks Balanced Salt Solution (BSS), pH 7.5. This was used free of calcium and magnesium to aid cell separation in subsequent procedures. Previous unpublished observations have shown that the exocoelomic fluid of the rabbit conceptus is rich in macrophages, as is the mesenchymal tissue of the paraplacental chorion<sup>5</sup>. In order to reduce possible contamination by macrophages and to provide a control population of Fc receptor positive cells, the exocoelomic fluid was removed by inserting a Pasteur pipette through a slit made in an avascular region of the chorion and flushing out the cavity with BSS. Flushing from different conceptuses were pooled. The yolk sac splanchnopleur was then dissected free of chorion, placed in a Petri dish with its endodermal surface uppermost and washed with BSS so as to remove blood issuing from the cut ends of vessels and macrophages possibly adhering to the exocoelomic mesothelium. BSS containing 0.5% trypsin and 10% foetal calf serum (FCS) was then added and incubation carried out at 37 °C for 1.5 h. The incubation medium was then carefully removed and fresh BSS/10% FCS pipetted on to the yolk sac endoderm so as to dislodge the cells. These were pooled from different conceptuses of the same rabbit and washed three times in a large volume of BSS/10% FCS. Between washings it was found necessary to pass cells through a hypodermic needle to achieve a single-cell suspension. Cells were finally suspended in Eagles Minimal Essential Medium containing 10% FCS and antibiotics at a concentration of  $5 \times 10^5$  cells per ml.

A variable but significant number of rosettes were formed between yolk sac endodermal cells and sheep red blood cells (SRBC) coated with rabbit IgG; control SRBC and SRBC coated with IgM, and with F(ab')<sub>2</sub> or Fab fragments of IgG, did not form rosettes (Table 1). These results therefore show the presence of a receptor for the Fc region of rabbit IgG on at least a proportion of yolk sac endodermal cells; the variability observed is similar to that found for mouse yolk sac endoderm<sup>1</sup>. A very high percentage of exocoel macrophages also formed Fc dependent rosettes but we are confident these were not the source of rosetting cells in yolk sac endodermal cell preparations. By observing and counting rosettes under high power phase contrast microscopy, yolk sac endodermal cell rosettes could readily

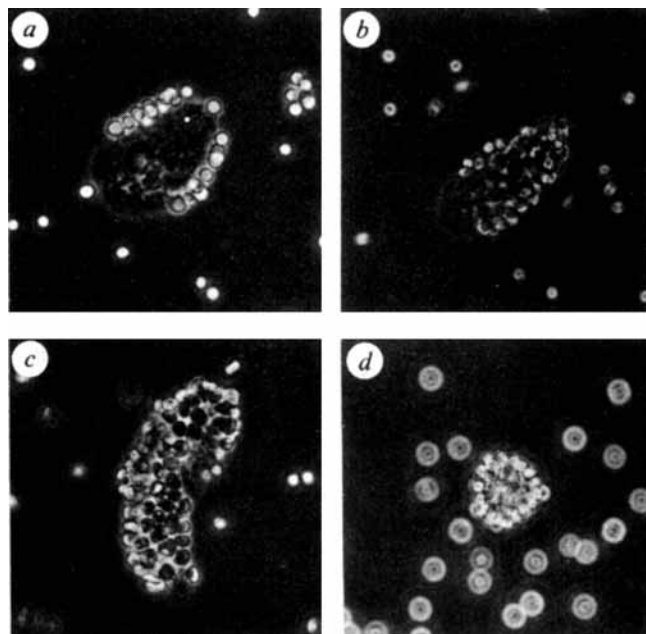


Fig. 1 Appearance of EA rosettes formed with yolk sac endodermal cells (a, b and c) and an exocoel macrophage (d). All photographs taken at the same initial magnification of  $\times 298$ .

be distinguished from those formed by exocoel macrophages by their difference in morphology. Rosetting yolk sac endodermal cells frequently retained their columnar shape and sensitised SRBC were seen attached either in a band mainly at one pole (Fig. 1a) or distributed more widely over the cell surface (Fig. 1b, c). Exocoel macrophage rosettes differed by being smaller and very round in appearance with attached SRBC covering the cell surface (Fig. 1d). Several factors could have influenced the proportion and also the type of rosetting yolk sac endodermal cells observed. Although there may well be genuine differences between endodermal cells with respect to whether or not they possess Fc receptors, receptor sites might also have been blocked on some cells by endogenous IgG present within the uterine lumen; such IgG might be differentially released by the action of trypsin. Longer exposures of yolk sac splanchnopleur to trypsin-containing incubation medium did not have any consistent effect on the percentage of yolk sac endodermal rosettes subsequently scored, but it has been reported that treatment of formalin-fixed yolk sac splanchnopleur with trypsin reduces subsequent specific rabbit IgG binding by about 75% (ref. 8). Movement of receptors at some stage after isolation of cells from what is normally a polarised epithelium, might explain why attachment of sensitised SRBC occurs at sites other than what may be construed as the apical cell surface. But, no increase in the number of cells with SRBC attached more widely over the cell surface was seen when cell suspensions were further incubated after initial rosette counts. In an alternative mechanism to that proposed by Brambell, it has been suggested<sup>9</sup> that the receptor functions not so as to protect immunoglobulin from proteolysis within phagolysosomes, but rather to segregate, at the cell surface, immunoglobulin destined for transport and that destined for proteolysis. It was envisaged that the receptor became associated with a distinct class of transporting coated micropinocytotic vesicles and subsequent studies<sup>10</sup> have detected rabbit and human IgG, but not bovine IgG, in coated vesicles in confluence with the lateral and basal plasmalemma. Thus endodermal cells engaged in transporting IgG may normally have free receptors generated at sites other than the apical cell surface as a result of coated vesicles fusing with the

Table 1 Effect of rabbit antibody class and fragments on EA rosette formation with yolk sac endoderm (YSE) and exocoel macrophages (ExMac)

| Sensitising antibody* | % Rosettes† obtained with YSE from animals 1–5 |    |    |      |    | % Rosettes† obtained with ExMac from animals 1–4 |     |     |    |  |
|-----------------------|------------------------------------------------|----|----|------|----|--------------------------------------------------|-----|-----|----|--|
| Whole serum           | 11                                             | 52 | 50 | 22.6 | 25 | 97                                               | 79  | 97  | 83 |  |
| IgG                   | 32.5                                           | NT | NT | 21.6 | 19 | 100                                              | NT  | 97  | NT |  |
| IgM                   | 0                                              | NT | NT | 0    | 0  | 0                                                | NT  | 7   | NT |  |
| F(ab') <sub>2</sub>   | 0                                              | 0  | 0  | 0    | 0  | 0                                                | 4   | 7   | 0  |  |
| Fab                   | 0                                              | 0  | 0  | 0    | 0  | 0                                                | 3.7 | 1.5 | 0  |  |
| Control SRBC          | 0                                              | 0  | 0  | 0    | 0  | 0                                                | 3.5 | 4   | 0  |  |

NT, Not tested

\*IgG, IgM, F(ab')<sub>2</sub> and Fab fractions derived from IgG were pure as defined by immunoelectrophoresis.

†Rosettes were formed by mixing equal volumes of YSE or ExMac and a 0.7% suspension of appropriately sensitised SRBC (tested for sensitisation with goat anti-rabbit immunoglobulins). Such mixtures were centrifuged at 150g for 3 min and the pellet gently resuspended. Aliquots were then mounted beneath coverslips on microscope slides and observed with  $\times 40$  phase objective. Cells showing at least five adherent SRBC were scored as positive. At least 100 cells were counted and the percentage of YSE or ExMac forming rosettes calculated. Duplicate samples normally showed agreement to within 5%.

plasma membrane and these might account for the wide distribution of attached SRBC.

Rabbit, human and bovine IgG solutions were tested at two different protein concentrations for their ability to inhibit attachment of rabbit IgG sensitised SRBC to yolk sac endodermal cells. Rabbit and human Ig were most effective in this respect, but little or no inhibition occurred with bovine Ig (Table 2). Human IgG and rabbit IgG Fc were also very effective in inhibiting attachment but human IgM and rabbit IgG Fab had very much less, and in some cases, no effect. Rabbit albumin was also ineffective; indeed for some unknown reason this consistently enhanced the number of yolk sac endodermal cells forming rosettes with

**Table 2** Inhibition of YSE-EA rosette formation by homologous and heterologous proteins

| Protein tested*<br>(mg ml <sup>-1</sup> ) | Mean %<br>inhibition†        | Range          | No. of<br>animals |
|-------------------------------------------|------------------------------|----------------|-------------------|
| Rabbit Ig                                 |                              |                |                   |
| 0.5                                       | 78.5                         | 68.0–83.3      | 4                 |
| 5.0                                       | 95.0                         | 90.0–100       | 3                 |
| Human Ig                                  |                              |                |                   |
| 0.5                                       | 93.4                         | 91.1–95.4      | 4                 |
| 5.0                                       | 100                          | 100–100        | 3                 |
| Bovine Ig                                 |                              |                |                   |
| 0.5                                       | 14                           | 0.0–28.0       | 4                 |
| 5.0                                       | (–)3.0                       | (–)14.3–17.1   | 3                 |
| Human IgG                                 | 85.9                         | 83.1–88.7      | 2                 |
| Human IgM                                 | all at 6.8                   | 0.0–13.6       | 2                 |
| Rabbit IgG Fc                             | 0.5 mg ml <sup>-1</sup> 71.4 | 60.2–78.5      | 3                 |
| Rabbit IgG Fab                            | final conc. 2.0              | (–)35.5–30.0   | 3                 |
| Rabbit albumin                            | (–)6.2                       | (–)2.2–(–)11.5 | 3                 |

YSE cells were mixed with equal volumes of protein solution and left at room temperature for 30 min before adding equal volumes of either sensitised or control SRBC. Rosettes were then enumerated as described in Table 1. All tests carried out with control SRBC were negative in these experiments.

\*Human and bovine Ig were obtained from Koch-Light (Cohn Fr. 2) and rabbit Ig from Calbiochem; they were principally IgG.

†% Inhibition = 100 – (% rosettes formed between IgG sensitised SRBC and YSE in presence of competing protein/% rosettes formed in absence of competing protein × 100).

sensitised SRBC. These results thus confirm the importance of the Fc region of IgG for attachment to receptors on yolk sac endoderm. They also indicate that rabbit and human IgG bind to the same Fc receptor but that little if any binding of bovine IgG, human IgM or rabbit serum albumin occurs to this same receptor. The possibility does of course exist that separate receptors are present for these non-competing proteins, but that this is not the case for bovine IgG was shown by immunising a cow with SRBC and sensitising the SRBC with antibodies present in whole serum or the IgG fraction of this serum. As can be seen from Table 3, bovine IgG sensitised SRBC, unlike rabbit IgG sensitised SRBC, do not form rosettes with yolk sac endodermal cells. This could not be attributed to lack of a comparable degree of sensitisation, since exocoel macrophages showed a similar high level of rosette formation in parallel studies carried out with bovine or rabbit IgG sensitised

**Table 3** Effect of rabbit and bovine IgG sensitising antibody on EA rosette formation with yolk sac endoderm (YSE) and exocoel macrophages (ExMac)

| Source of sensitising<br>antibody to SRBC | Mean % rosettes*<br>for YSE<br>and range | Mean % rosettes*<br>for ExMac<br>and range | No. of<br>animals |
|-------------------------------------------|------------------------------------------|--------------------------------------------|-------------------|
| Rabbit whole serum                        | 11.8 (8.0–19.0)                          | 97.0 (94.0–100)                            | 4                 |
| Rabbit IgG fraction                       | 26.3 (16.0–45.5)                         | 81.6 (52.0–96.5)                           | 6                 |
| Bovine whole serum                        | 0 (0)                                    | 56.2 (25.0–100)                            | 4                 |
| Bovine IgG fraction                       | 0.2 (0–1.0)                              | 75.8 (50.0–96.0)                           | 6                 |
| Control SRBC                              | 0.2 (0–2.0)                              | 3.5 (0–11.0)                               | 10                |

\*Determined as described in Table 1.

SRBC. As mentioned above, bovine IgG is not transported across yolk sac endoderm to any significant extent, although it is readily endocytosed. Rabbit and human IgG are more readily transported across rabbit yolk sac endoderm and all three immunoglobulins can be detected in phagolysosomes<sup>11</sup>. Since there is no apparent binding of bovine IgG to an Fc receptor, the inescapable conclusion seems to be that there is an association between the Fc receptor and selective immunoglobulin transport. Clearly, yolk sac endodermal cells, in view of their difference in binding affinity for bovine IgG, must have subtle differences in the nature of the Fc receptor compared with that present on macrophages. The presence of Fc receptors specific for IgG has also been demonstrated on human syncytiotrophoblast, both by EA rosette formation<sup>12</sup> and immune cytoadherence<sup>13</sup>. Also, binding of IgG to the human placental receptor seems to be dependent on an intact Fc region since neither C2 or C3 homology regions would competitively inhibit binding of IgG (ref. 14). This is again different to the macrophage Fc receptor, which will bind the isolated C3 homology region<sup>15</sup>.

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## Localisation of gonadotropin-releasing hormone in the circumventricular organs of human brain

GONADOTROPIN-releasing hormone (GnRH) and thyrotropin-releasing hormone (TRH) are two hypothalamic oligopeptides which regulate the secretion of gonadotropins and thyrotropin, respectively, from the anterior pituitary<sup>1,2</sup>. It is generally accepted that these neurohormones are produced by nerve cells, transmitted along axons and stored in nerve endings. When released, they are transferred by way of the hypothalamo-hypophyseal portal system to the anterior pituitary<sup>3</sup>. We have recently reported<sup>4</sup> that in the human hypothalamus most of the GnRH is found in the preoptic area while TRH is localised mainly in the posterior nucleus. Exceedingly high concentrations of the two neurohormones were found in the pituitary stalk. The localisation of the two neurohormones in distinctive regions of the hypothalamus and pituitary stalk may represent sites of synthesis in neuronal cell bodies and storage in nerve terminals, respectively. It has recently been found<sup>5</sup> that significant amounts of GnRH are present in the circumventricular organs (CVO) of the rat brain. These organs consist of specialised ependymal cells, small nerve cells and axon terminals containing small dense core vesicles. The

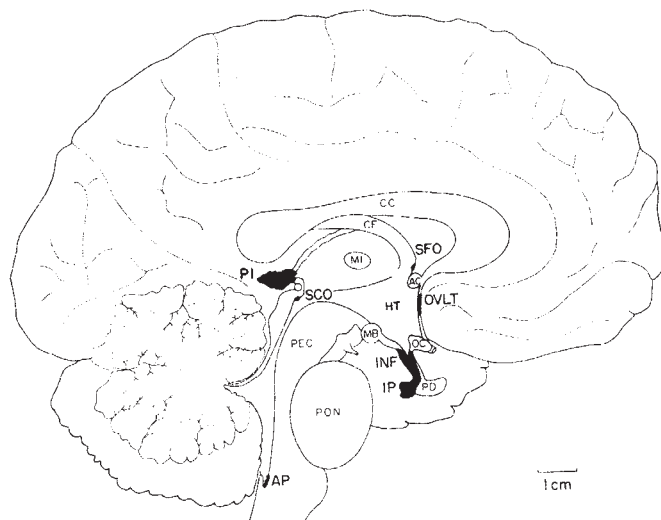


axon terminals end on fenestrated capillaries comprising many capillary loops<sup>6</sup>. Such an anatomical arrangement is characteristic of neurohumoral secretion, and is present also in the human pituitary stalk<sup>7</sup>. In this study we have determined, by radioimmunoassay, the distribution of GnRH and of TRH in the circumventricular organs of the human brain. We report that exceedingly high concentrations of GnRH, but not of TRH, are present in one of the circumventricular organs, the organum vasculosum of the lamina terminalis (OVLT), and discuss the possible physiological function of this structure in the neural control of reproduction.

Different brain structures were removed during routine autopsies carried out at the Institute of Forensic Medicine, Tel Aviv. The tissues were obtained from six females, 19–70 yr old, and from four 30–70-yr-old males, seven of them killed in road accidents and three suicide cases. The bodies were kept for 4–52 h at 4 °C until autopsy. Identical storage conditions did not affect GnRH or TRH content of brain tissue<sup>4</sup>. The circumventricular organs (Fig. 1) and the tissues surrounding them were removed under a dissecting microscope and sectioned into several blocks. The pituitary stalk and specific hypothalamic regions were sectioned as described previously<sup>4</sup>. Each of the segments (52 tissue blocks per brain) was extracted and analysed for GnRH and TRH by radioimmunoassay, as described previously<sup>8,9</sup>. Protein determination was performed according to the Lowry method<sup>10</sup>. The pineal body, a circumventricular organ, was excluded from this study since we have previously shown that it does not contain any significant amount of GnRH or TRH (ref. 4).

The largest amount of GnRH and TRH was found in the pituitary stalk (52 ng of GnRH and 21 ng of TRH). A considerable amount, however, of GnRH (9.4 ng) was also found in the OVLT (Table 1); its concentration in this structure (36.4 ng per mg protein), exceeded even that found in the pituitary stalk (20.7 ng per mg protein). The lamina terminalis surrounding the vascular structure, as well as the other CVO, contained negligible amounts of GnRH. Relatively low concentrations of TRH were found in all the CVO (Table 1). Within this limited series, neither the amount and concentration of GnRH in the OVLT and pituitary stalk, nor the distribution of the neurohormone between these two organs could be shown to be related to age or sex of the subjects.

It seems likely that in the human, the OVLT, like the pituitary stalk, functions as a neurosecretory organ: the specific localisation and high concentration of GnRH in both organs, and the presence of a rich vasculature, and of nerve endings terminating on capillary loops, would suggest neurohumoral secretion of the hormone at these two sites. The physiological significance of GnRH secretion at the pituitary stalk is well established: the hormone is carried by way of the hypothalamo-hypophyseal portal system to the anterior pituitary, thereby inducing gonadotropin release. The role of GnRH originating in the OVLT is not



**Fig. 1** The circumventricular organs of the human brain (in black), in a midsagittal plane section. AP, area postrema; INF, infundibulum of neurohypophysis (pituitary stalk); IP, infundibular process of neurohypophysis (posterior pituitary); OVLT, organum vasculosum lamina terminalis; PI, pineal body; SCO, subcommissural organ; SFO, subfornical organ. Other structures: AC, anterior commissure; CC, corpus callosum; CF, column of fornix; HT, hypothalamus (shaded area); MB, mammillary body; MI, massa intermedia; OC, optic chiasma; PD, pars distalis of adenohypophysis; PEC, peduncle of cerebellum; PON, pons.

clear. Transport of the hormone to the anterior pituitary by the circuitous route of the cerebrospinal fluid or by the general circulation seems unlikely. Alternatively, the drainage of the OVLT towards the preoptic area in the human<sup>11</sup> could provide a pathway for distribution of GnRH to diverse brain regions. In these areas, the hormone could modulate neurotransmitter functions or influence the activity of different neurones.

Several observations suggest a possible relationship between the OVLT and behavioural and endocrine parameters of sexual function. Destruction of the preoptic area, to which the OVLT is adjacent, modifies sexual behaviour in several animal species<sup>12</sup> and disrupts the female oestrous cycle of the rat<sup>13</sup>. Wenger<sup>14</sup> has recently shown a correlation between the endocrine state of the female rat and the number of dense core vesicles in the OVLT resembling those of the GnRH-containing vesicles of the median eminence of the hypothalamus. Moreover, a direct effect of GnRH on sexual behaviour has been demonstrated by Moss and McCann<sup>15</sup> and by Pfaff<sup>16</sup>, who by administration of GnRH to ovariectomised or ovariectomised hypophysectomised rats were able to induce mating behaviour.

While in the rat brain considerable concentrations of GnRH were found in all the CVO (ref. 5), the human brain

**Table 1** GnRH and TRH in the CVO of the human brain

| Structure                           | Content (ng)  | GnRH                              | TRH                               |
|-------------------------------------|---------------|-----------------------------------|-----------------------------------|
|                                     |               | Concentration (ng per mg protein) | Concentration (ng per mg protein) |
| Pituitary stalk                     | 51.98 ± 9.69* | 20.75 ± 1.62 <sup>†</sup>         | 21.30 ± 3.38                      |
| Vascular organ of lamina terminalis | 9.42 ± 1.67   | 36.40 ± 5.22                      | 1.31 ± 0.69                       |
| Subfornical organ                   | 0.85 ± 0.26   | 1.21 ± 0.37                       | 0.64 ± 0.18                       |
| Subcommissural organ                | 0.35 ± 0.20   | 0.50 ± 0.28                       | 0.37 ± 0.20                       |
| Area postrema                       | 0.13 ± 0.11   | 0.18 ± 0.15                       | 0.74 ± 0.13                       |
| Preoptic area of hypothalamus       | 4.78 ± 0.67‡  | 1.86 ± 0.51                       | 3.92 ± 0.31‡                      |
| Cerebral cortex                     |               | < 0.03                            | 0.06 ± 0.04                       |

\*Mean ± s.e.m. for 10 samples are shown.

†Peak concentrations of GnRH and TRH in the upper portion of the pituitary stalk.

‡Content of the POA of half a hypothalamus, dissected in the midsagittal plane.

contains high concentrations of GnRH in the pituitary stalk and OVLT only. The basic anatomical resemblance of the OVLT and the pituitary stalk suggests that GnRH is secreted from the vascular organ as well. It is possible that the GnRH secreted from the OVLT may have a role in the regulation of the menstrual cycle and sexual behaviour. However, further work is required to establish the exact physiological role of GnRH localised in the OVLT.

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## Chemical carcinogens stimulate canine kidney (MDCK) cells to produce prostaglandins

WHILE investigating the regulation of prostaglandin production by cells, I found that the MDCK line from dog kidney<sup>1</sup> responds to treatment with several polycyclic aromatic hydrocarbon carcinogens, aflatoxin B<sub>1</sub> and 1,2,7,8-diepoxyoctane. Metabolic activation of the hydrocarbons was required for this stimulation of prostaglandin production.

The MDCK cell line, which morphologically resembles epithelium, was obtained from Dr Charles D. Stiles, University of California, San Diego. Exponentially growing cells were incubated in Eagle's minimal essential medium containing 2 mM L-glutamate and supplemented with 10% (v/v) foetal bovine serum and penicillin-streptomycin. Duplicate dishes were used for each reagent and each experiment was repeated at least once. The values obtained for prostaglandins per cell were similar for each reagent in all experiments. Media were assayed for PGE<sub>2</sub> and PGF<sub>2α</sub> with either anti-PGE<sub>2</sub> or anti-PGF<sub>2α</sub>: anti-PGF<sub>2α</sub> reacts 0.1% with PGE<sub>2</sub> and the anti-PGE<sub>2</sub> reacts 0.01% with PGF<sub>2α</sub>. (The antisera do not identify the prostaglandins as either monoenoic or dienoic.)

MDCK cells produce prostaglandins—about eight times more PGF<sub>2α</sub> than PGE<sub>2</sub>. When the cells were incubated in the presence of benzo(a)pyrene (0.1 μg ml<sup>-1</sup>), the content of PGF<sub>2α</sub> and PGE<sub>2</sub> in the medium increased. In my experiments the production of prostaglandins by MDCK cells and its stimulation in the presence of benzo(a)pyrene depended on cell density. At all densities, however, the cells produced greater levels of prostaglandins in the presence of benzo(a)pyrene (0.1 μg ml<sup>-1</sup>).

When cells were plated at 2 × 10<sup>5</sup> per dish, stimulation by benzo(a)pyrene (0.1 μg ml<sup>-1</sup>) was observed after 48 h of incubation. Benzo(a)pyrene was not required throughout the 48 h. There was equivalent stimulation if cells were incubated at

2 × 10<sup>5</sup> per dish for 24 h with benzo(a)pyrene, washed twice and incubated for a further 24 h in medium lacking benzo(a)pyrene. This procedure therefore was used to test the various reagents. Medium from the second 24-h incubation was assayed for serological activity with anti-PGF<sub>2α</sub> and anti-PGE<sub>2</sub>.

Table 1 shows that the cells incubated with the most active polycyclic aromatic carcinogens<sup>2–6</sup> produced the highest levels of prostaglandins. Incubation with benzo(a)pyrene at 0.5 μg ml<sup>-1</sup> stimulated prostaglandin production 13-fold and at 0.2 μg ml<sup>-1</sup>, 5–5.5-fold. Among the fungal toxins tested, only the most potent carcinogen, aflatoxin B<sub>1</sub> (ref. 5) at 0.05 and 0.02 μg ml<sup>-1</sup>, stimulated prostaglandin production, while the weakly active carcinogenic aflatoxins, at 0.1 μg ml<sup>-1</sup>, did not. Neither the aromatic amines nor the nitrosamines, at 0.5 μg ml<sup>-1</sup>, affected prostaglandin production.

The direct-acting carcinogen, 1,2,7,8-diepoxyoctane, at 0.5 μg ml<sup>-1</sup>, stimulated prostaglandin production 3–3.5-fold. The 1.7-fold stimulation due to chrysene (0.5 μg ml<sup>-1</sup>) was statistically significant (0.533 ± 0.05 fg of PGE<sub>2</sub> per cell (medium) and 0.903 ± 0.19 fg per cell (chrysene) *P* < 0.005; and 2.92 ± 0.26 fg PGF<sub>2α</sub> per cell (medium) and 4.93 ± 0.48 fg per cell (chrysene) *P* < 0.005).

Table 1 Prostaglandin production by MDCK cells

| Effector*                          | Increase in prostaglandin production |                     | Carcinogenicity |
|------------------------------------|--------------------------------------|---------------------|-----------------|
|                                    | PGE <sub>2</sub> †                   | PGF <sub>2α</sub> † |                 |
| Medium (control)                   | 1.0                                  | 1.0                 | —               |
| Benzo(a)pyrene                     | 13.6                                 | 12.2                | +               |
| Benzo(a)pyrene§                    | 5.1                                  | 5.7                 | +               |
| 7,12-Dimethylbenz(a)anthracene     | 10.9                                 | 10.6                | +               |
| 3-Methylcholanthrene               | 4.1                                  | 4.8                 | +               |
| 7-Methylbenzanthracene             | 4.2                                  | 3.9                 | +               |
| 5-Methylchrysene                   | 3.6                                  | 3.8                 | +               |
| 3-Methylchrysene                   | 2.0                                  | 2.1                 | +               |
| Chrysene                           | 1.7                                  | 1.7                 | +               |
| 1-Methylchrysene                   | 1.4                                  | 1.6                 | —               |
| Dibenz(a,h)anthracene              | 1.0                                  | 1.4                 | +               |
| Benz(g,h,i)perylene                | 1.2                                  | 1.1                 | +               |
| Benz(a)anthracene                  | 1.2                                  | 1.5                 | +               |
| 1,2,3,4-Dibenzanthracene           | 1.0                                  | 1.0                 | —               |
| Anthracene                         | 0.8                                  | 0.9                 | —               |
| Phenanthrene                       | 0.7                                  | 0.3                 | —               |
| Pyrene                             | 0.8                                  | 1.1                 | —               |
| Perylene                           | 0.8                                  | 1.2                 | —               |
| Aflatoxin B <sub>1</sub> ¶         | 4.6                                  | 5.0                 | +               |
| Aflatoxin B <sub>1</sub> ‡         | 2.6                                  | 2.2                 | +               |
| Aflatoxin B <sub>2</sub> **        | 0.7                                  | 1.0                 | +               |
| Aflatoxin G <sub>1</sub> **        | 1.0                                  | 1.2                 | +               |
| Aflatoxin G <sub>2</sub> **        | 1.2                                  | 1.3                 | +               |
| 2-Aminoanthracene                  | 1.3                                  | 1.3                 | +               |
| 2-Aminofluorene                    | 0.9                                  | 1.0                 | +               |
| 6-Aminochrysene                    | 1.7                                  | 1.5                 | +               |
| 2-Acetoaminofluorene               | 1.5                                  | 1.3                 | +               |
| 4-Aminobiphenyl                    | 1.1                                  | 1.2                 | +               |
| Dimethylnitrosamine                | 1.2                                  | 1.1                 | +               |
| Diethylnitrosamine                 | 1.1                                  | 1.2                 | +               |
| Dibutylnitrosamine                 | 1.5                                  | 1.6                 | +               |
| 1,2,7,8-Diepoxyoctane              | 3.5                                  | 3.0                 | +               |
| 5,6-Benzoflavone**                 | 1.1                                  | 1.2                 | —               |
| 7,8-Benzoflavone**                 | 1.2                                  | 1.3                 | —               |
| SKF 525-A††                        | 1.2                                  | 1.0                 | —               |
| Metirapone††                       | 1.3                                  | 1.0                 | —               |
| 1,2-Epoxy 3,3,3-trichloropropane†† | 1.2                                  | 1.1                 | —               |
| Nicotine                           | 1.0                                  | 1.1                 | —               |
| Phenobarbital‡‡                    | 0.9                                  | 1.0                 | —               |
| Dimethylsulphoxide§§               | 1.0                                  | 1.1                 | —               |

\*Unless specifically noted, 0.5 μg ml<sup>-1</sup> was used.

†Expressed as relative to level measured after incubation in medium alone (1.0); for example, a value of 13.6 represents a 13.6-fold increase in prostaglandin production. Carcinogenic activities obtained from the literature<sup>2–6</sup>.

§0.2 μg ml<sup>-1</sup>.

¶0.05 μg ml<sup>-1</sup>.

‡0.02 μg ml<sup>-1</sup>.

\*\*0.1 μg ml<sup>-1</sup>.

††1.0 μg ml<sup>-1</sup>.

‡‡10.0 μg ml<sup>-1</sup>.

§§0.05%.



Several polycyclic aromatic hydrocarbons stimulated prostaglandin production. Metabolism of these compounds to arene oxide products by enzymes such as aryl hydrocarbon hydroxylases are required for carcinogenic activity<sup>6,7</sup>. Such metabolism can be blocked by 7,8-benzoflavone<sup>8,9</sup>. 7,8-Benzoflavone inhibited stimulation by benzo(a)pyrene, 7,12-dimethylbenz(a)anthracene and aflatoxin B<sub>1</sub>, but not that of 1,2,7,8-diepoxyoctane; 7,8-benzoflavone, on the other hand, increased stimulation by the latter (Table 2). The inhibitors of microsomal monooxygenases, SKF 525-A and metyrapone<sup>10</sup> (0.1 µg ml<sup>-1</sup>) inhibited stimulation by both benzo(a)pyrene and 1,2,7,8-diepoxyoctane by 20%; while 1,2-epoxy 3,3,3-trichloropropane (1.0 µg ml<sup>-1</sup>), an inhibitor of epoxide hydrolase, had no effect.

**Table 2** Inhibition by 7,8-benzoflavone of stimulation of prostaglandin production in MDCK cells by benzo(a)pyrene

| Effector                       | µg ml <sup>-1</sup> | PGF <sub>2α</sub><br>(fg per cell) | Increase in<br>prostaglandin<br>production* |
|--------------------------------|---------------------|------------------------------------|---------------------------------------------|
| No addition                    | —                   | 3.9                                | 1.0                                         |
| 7,8-Benzoflavone               | 0.1                 | 3.6                                | 0.9                                         |
| 7,8-Benzoflavone               | 0.01                | 3.7                                | 0.9                                         |
| 7,8-Benzoflavone               | 0.001               | 3.8                                | 1.0                                         |
| Benzo(a)pyrene                 | 0.1                 | 16.7                               | 4.3                                         |
| + 7,8-Benzoflavone             | 0.1                 | 4.1                                | 1.1                                         |
| + 7,8-Benzoflavone             | 0.01                | 9.4                                | 2.4                                         |
| + 7,8-Benzoflavone             | 0.001               | 16.8                               | 4.3                                         |
| 7,12-Dimethylbenz(a)anthracene | 0.5                 | 40.1                               | 10.3                                        |
| + 7,8-Benzoflavone             | 0.1                 | 9.0                                | 2.3                                         |
| Aflatoxin B <sub>1</sub>       | 0.05                | 16.4                               | 4.2                                         |
| + 7,8-Benzoflavone             | 0.1                 | 6.2                                | 1.6                                         |
| 1,2,7,8-Diepoxyoctane          | 0.5                 | 11.7                               | 3.0                                         |
| + 7,8-Benzoflavone             | 0.1                 | 19.9                               | 5.1                                         |

\*Expressed as relative to level found in medium alone (1.0); for example, a value of 4.3 represents a 4.3-fold increase in prostaglandin production.

After incubation with the carcinogens that stimulated prostaglandin production, trypsin-treated MDCK cells were larger than either trypsin-treated control cell or cells incubated with non-stimulating reagents. No attempt, however, was made quantitatively to correlate this increase in cell size with increased prostaglandin production.

Greater prostaglandin production by transformed cell lines than by their parent cell lines has been reported<sup>12,13</sup>. Methylcholanthrene-transformed mouse fibroblasts produce more prostaglandins because their synthetase activity is higher<sup>13</sup>. Stimulation of the activity of the microsomal enzyme complex prostaglandin synthetase could also account for the stimulation reported here. On the other hand, increased synthetase activity may reflect the morphological changes in the cells, with no induction of enzyme. Increased phospholipase activity, either as a result of induction of phospholipases or as a consequence of the morphological alterations, could also account for the stimulated prostaglandin production by increasing the availability of arachidonic acid, the substrate for prostaglandin synthetase.

Human fibroblasts (D550), rabbit endothelial cells (CLO) and mouse fibroblasts (3T3), although they produce prostaglandins, were unaffected by benzo(a)pyrene (0.2 µg ml<sup>-1</sup>; 48 h incubation). MDCK cells probably contain the metabolic enzymes necessary to convert the procarcinogens to prostaglandin-stimulating compounds, but they do not seem to have those required to activate aromatic amines or nitrosamines to stimulators of prostaglandin production; other cell lines which may have these enzymes are being studied. The relationship of carcinogenicity to stimulation of prostaglandin production at the concentrations used is positive (Table 1). With increased concentrations of some of the reagents (5 µg ml<sup>-1</sup> and above), the false negatives may be positive. But toxicity becomes marked at higher levels and a procedure to quantify the contributions of the toxic and non-toxic effects to prostaglandin production is being developed. The MDCK cell line may prove to be a

sensitive indicator of the carcinogenic potential of the hydrocarbons.

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## Infectious primate type-C virus shed by healthy gibbons

MANY captive gibbons are naturally exposed to infectious type-C virus based on the prevalence of animals with humoral antibodies reactive to the virus<sup>1,2</sup> and isolation of virus from tissue of leukaemic<sup>3,4</sup> and normal gibbons<sup>5</sup>. A closely related infectious type-C virus has also been isolated from a woolly monkey with a spontaneous fibrosarcoma<sup>6,7</sup> which implies that a common virus could infect unrelated non-human primates. Several reports recently have identified infectious primate type-C virus<sup>8,9</sup>, viral components<sup>10</sup> and humoral antibody reactive to this virus in leukaemic<sup>11</sup> and normal humans<sup>12</sup> suggesting that man also may be susceptible to infection by a related virus. In an attempt to identify a possible source for infectious primate type-C virus that might infect primates as well as other animals, we decided to investigate whether healthy captive gibbons were shedding virus since this non-human primate is frequently displayed at zoos or kept as pets. This report presents evidence that gibbons can have long term viraemia with type-C virus without detectable immune response. These viraemic animals actively shed infectious virus in urine and faeces.

Blood samples were taken monthly or when available from laboratory, pet and zoo gibbons (*Hylobates* sp.) for clinical, virological and serological studies. All gibbons under observation were initially free of any clinical symptoms. Blood samples (3-5 ml) were taken from the femoral vein for plasma and sera. Plasma was used to infect virus-susceptible cell cultures. Serum samples were used to detect the presence of humoral antibody.

Faecal and urine specimens were collected during the monthly physical examination. Each urine specimen was filtered through a 0.45 µm Millipore filter then concentrated by differential centrifugation. The sterile concentrate was resuspended in physiological buffered saline (PBS) and used as an inoculum. Each faecal sample was initially diluted as a 20% suspension in PBS. The suspension was subjected to low speed centrifugation to remove the large particles. The clarified suspension was filtered through a 0.45 µm Millipore filter, then subjected to high speed centrifugation to pellet the virus. The pellet was resuspended in PBS and used for inoculum.

Fifty-three gibbons were examined over 12 months for the presence of infectious type-C virus and viral-specific humoral antibody. All gibbons were separated into two major groups depending on whether the animals were or were not exposed to the virus. Animals in group 1 were considered normal, that is,

free of detectable infectious virus and humoral antibody. Animals in group 2 were those animals which had been exposed to type-C virus as determined by either the presence of infectious virus or humoral antibody. Results are summarised on Table 1. Thirty-three of the 53 gibbons (62%) examined were considered normal. Two hundred and ten plasma samples from these animals lacked evidence for the presence of infectious virus and humoral antibody. None of these animals seroconverted during the study period.

Animals in group 2 were divided into two subgroups based on whether the animal had detectable humoral antibody or infectious viraemia. Two hundred and twenty-two blood samples from 14 antibody positive gibbons were examined for humoral antibody and virus. All the samples from subgroup A contained humoral antibody but none of the samples contained detectable infectious virus. The humoral antibody titre for viral envelope antigen in these animals generally ranged from 1/4 to 1/16 by IFA except for one animal which ranged from 1/32 to 1/128. The radioimmune precipitation assay for P<sub>30</sub> ranged from 1/50 to 1/490 (ref. 14). Many of these animals were examined on several later occasions and in no instance did any of them convert from antibody positive to detectable viraemia. Tests on 38 samples from six viraemic gibbons (subgroup B), however,

Table 1 Gibbon antibody and virus detection

| Group               | Animals | Antibody | Virus |
|---------------------|---------|----------|-------|
| 1 Normal            | 33      | 0/210    | 0/210 |
| 2 Virus-infected    |         |          |       |
| A Antibody-positive | 14      | 220/220  | 0/210 |
| B Antibody-negative | 6       | 0/38     | 38/38 |

Detection of virus was performed by infecting the susceptible human lymphoid cell culture (NC-37) with the prepared inoculum. Approximately  $2 \times 10^6$  viable cells were concentrated into a pellet and resuspended in 0.2 ml of inoculum. The suspension was agitated for 90 min and then fresh medium was added to each tube. After three passages (1:3) the virus was concentrated from the tissue culture supernatant and assayed for viral DNA-polymerase in the presence of oligo dT-poly rA<sup>3</sup>. Viable cells were examined for viral envelope antigen by direct immunofluorescence (IFA) as previously described<sup>4</sup>. All positive cell cultures were confirmed for replicating type C virions by electron microscopy<sup>3</sup>.

Antibody reactive to type-C virus in gibbon sera was determined by indirect immunofluorescence assay (IFA) using viable infected and non-infected gibbon and human lymphoid cells as targets. The secondary antibody was FITC conjugated goat antihuman IgG or anti-monkey IgG as previously described<sup>4</sup>.

showed that all the samples contained infectious type-C virus but lacked detectable humoral antibody. One viraemic gibbon has been observed for over 4 yr and some have been observed for at least 1 yr. Four of the six gibbons are still under observation, two died after 6 and 12 month observations. One animal developed malignant leukaemia-lymphoma while the other died of unknown cause. All the remaining gibbons continue to be in good health.

Isolation of virus from viraemic gibbons was consistent which suggested that these animals probably had a high virus titre. In order to determine the approximate titre in the plasma, infectious virus was titrated by the endpoint dilution method. Results in Table 2 indicate that most gibbons had high virus titre. One animal (HLA-21), which was examined many times, showed a constant titre between  $10^1$  and  $10^2$  infectious virus (IV) ml<sup>-1</sup>. These results indicate that viraemic gibbons can tolerate a very high virus titre without any overt ill effects.

Since infectious type-C virus could be consistently isolated from the blood of these viraemic gibbons, we examined urine and faecal samples from these animals to determine if infectious virus was being excreted which could serve as a possible source for dissemination of the agent. Results are summarised in Table 2. Infectious virus could be readily isolated from fresh urine and intermittently from faecal samples of viraemic gibbons. Isolation of infectious virus from excretory products seems to depend on the virus titre of the viraemic animals. None of the

normal nor antibody-positive gibbons was found to excrete detectable levels of infectious virus in their urine or faeces.

The present study clearly indicates that healthy gibbons can have persistent type-C virus infection and can shed infectious virus. Gibbons must be highly susceptible to type-C infection in order to account for the prevalence of the virus found in this species. In most instances, the animal developed an active immunity probably resulting from low grade chronic multiplication of the virus in the tissues. A few animals with high level virus multiplication developed long term viraemia without detectable humoral antibody response, possibly because of partial immunological tolerance for the virus or because the virus multiplication exceeded the antibody response in the host.

Table 2 Comparison of viraemia and detection of virus in urine and faeces

| Animal no. | Age (yr) | Health                       | Virus               | Detection of infectious virus |        |
|------------|----------|------------------------------|---------------------|-------------------------------|--------|
|            |          |                              | IV ml <sup>-1</sup> | Urine                         | Faeces |
| HLA-21     | 6        | normal                       | $10^{1-2}$          | 1/6                           | 0/3    |
| HLA-30     | 6        | malignant leukaemia-lymphoma | $10^4$              | ND                            | ND     |
| HLA-68     | 3.5      | normal                       | $10^5$              | 10/14                         | 4/14   |
| HLA-71     | 9        | normal                       | $10^5$              | 14/16                         | 10/16  |
| HLA-72     | 2        | normal                       | $10^6$              | 2/5                           | 4/5    |
| 4 AB pos   | 4-6      | normal                       | 0                   | 0/64                          | 0/70   |

This situation may be similar to cats infected with feline leukaemia virus (FeLV) where the infected cats lack diseases or detectable immune response<sup>15</sup>.

The gibbon virus is essentially identical to the woolly monkey fibrosarcoma virus<sup>16,17</sup> which has been demonstrated to be oncogenic in marmosets<sup>18</sup> and has characteristics in common with other known animal oncornaviruses. Based on molecular hybridisation analysis the infectious primate virus is exogenous to the species<sup>19,20</sup> and probably transmitted horizontally. The mode of transmission may be similar to FeLV<sup>21</sup>. While several investigators have reported detection or isolation of infectious primate type-C virus<sup>8,9</sup> and humoral antibody reactive to the agent in normal and leukaemic humans<sup>11,12</sup>, other investigators were unable to confirm these findings<sup>21</sup>. This controversial point requires further study and needs to be resolved. Although there is no evidence that the gibbon agent is infectious to man, it is known that many non-human primate agents can infect man<sup>23</sup>. More importantly, this study indicates that there are gibbons which seem to be in good health in zoo and pet environment which are shedding infectious type-C virus and may be a source for dissemination of this virus. Four gibbons in this study were from such an environment and were essentially normal in their behaviour as far as appetite and activity. Routine clinical and physical examination of these animals showed no discernible abnormalities and only with specific immunological tests was the infection detected. One gibbon developed malignant leukaemia-lymphoma during this study which suggests the oncogenicity of this agent. The other viraemic gibbons may develop similar disease, however, at present they continue to be healthy and active. Further studies are needed to determine whether the gibbon agent is infectious and oncogenic to other primates.

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## Spatial summation and light adaptation in the goldfish visual system

IN attempting to explain how visual acuity improves as illumination increases, Pirenne and Denton<sup>1</sup> suggested that the retinal image is sampled by numerous units, each summing the effects of light over a region of the retina. At very low luminances, only units with the largest summation areas are functional, but with increasing luminance smaller units come into play as their thresholds are exceeded. Since that suggestion was made, cells at all levels of the visual system have been studied electrophysiologically and many receptive fields have been described that might form the basis for such units. Goldfish and other cyprinid fishes, have been the subjects of many such studies, and their retinal synaptic connections have been revealed in detail. I report here some psychophysical experiments which show the effects of light adaptation on spatial summation in the goldfish. The results are consistent with the connections made by rods and cones in the cyprinid retina, and with the theory of a progressive recruitment of ganglion cells with smaller receptive field centres as background luminance is raised.

Radiance thresholds of goldfish (12 cm standard length) were measured by classical conditioning of respiration<sup>2</sup>. Each fish was restrained with its body parallel to a diffusing screen at 14 cm from the left eye. The test stimuli were disks of monochromatic light (533 nm) projected for 5 s in the centre of the fish's visual field. The animals were trained by giving a brief electric shock at the offset of the test stimulus so that the shock-elicited inhibition of breathing came to be evoked by the light stimulus. The occurrence of a response was judged by a 4-alternative forced choice procedure: the test stimulus was presented randomly in one of four 5-s periods within each minute. A response was counted if the stimulus period contained an interbreath interval longer than those in the other three periods. Thresholds were estimated from a staircase tracking procedure<sup>3</sup>.

Figure 1 shows how threshold radiance changes with stimulus diameter when dark adapted (a), and against an adapting tungsten-light background of 3.4 mL (b). When light flux is summed perfectly, sensitivity (1/threshold) increases in proportion to stimulus area (Ricco's Law) up to the limit of full summation, and thereafter increases no further. Since the data can be fitted by two straight line segments, one of slope 2, representing Ricco's Law, and one of zero slope, the limit of

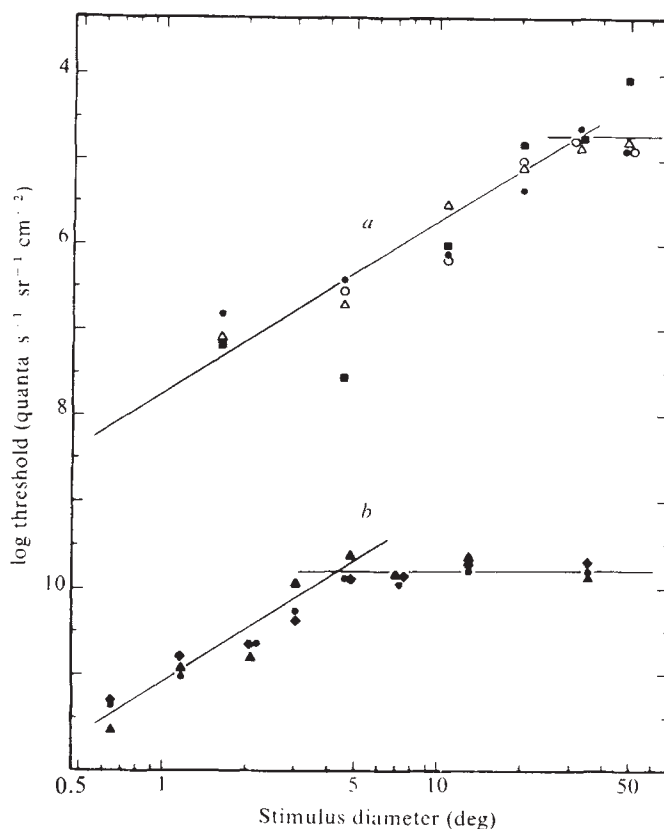


Fig. 1 Threshold stimulus radiance of wavelength 533 nm as a function of stimulus diameter at two different adaptation levels. Each symbol represents data from a different fish. The sloping lines show a reciprocal relationship between threshold and stimulus area (Ricco's Law). Lines fitted to data by eye. a, Thresholds obtained after at least 30 min of dark adaptation; b, thresholds to test stimuli projected on a tungsten-light background (3,000 K) of 3.4 mL.

full summation can be conveniently expressed by the 'critical diameter' (CD) at which the two lines intersect.

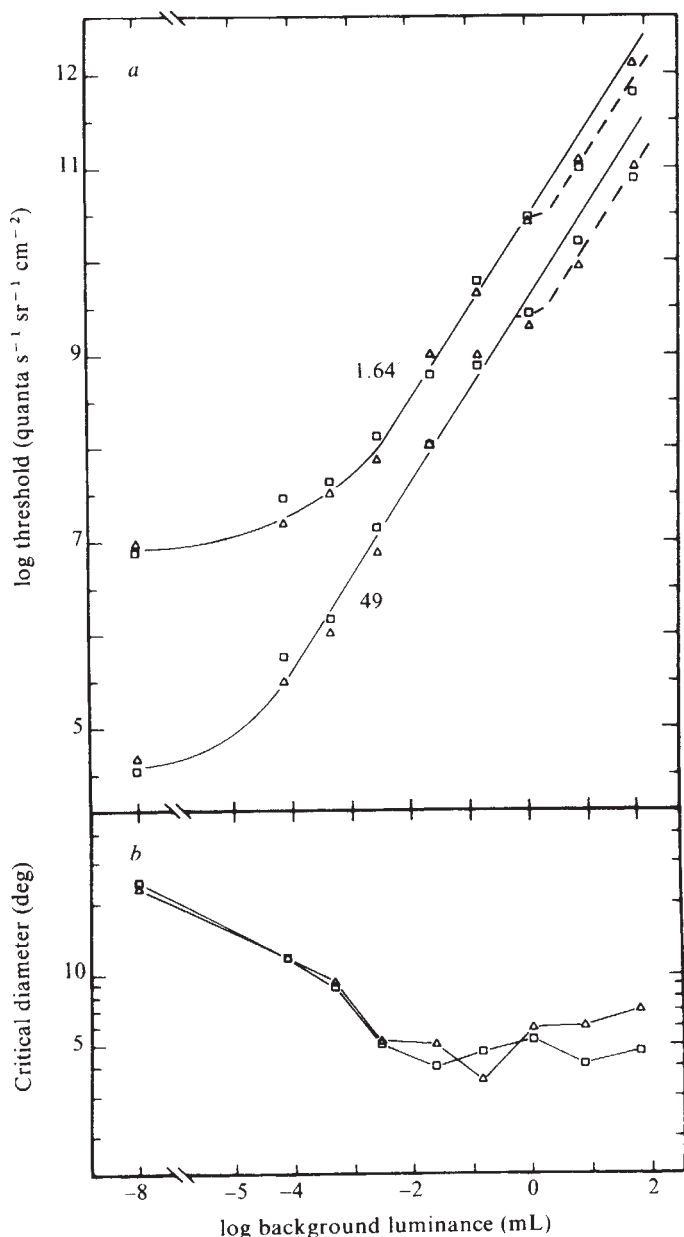
In the dark-adapted state, full summation takes place for stimuli subtending up to about 30° of visual angle. With a 49° diameter stimulus, the mean absolute threshold of four goldfish in terms of stimulus radiance was 58,200 quanta  $s^{-1} sr^{-1} cm^{-2}$ , which is 38% of the human absolute threshold measured in similar conditions<sup>4</sup>. Using targets of more than 100°, goldfish thresholds fell to about 15% of the human value, indicating some partial summation above 30°. Very similar absolute thresholds to large stimuli have previously been obtained for goldfish<sup>5</sup>.

To examine how sensitivity and spatial summation vary with the state of adaptation, thresholds in two goldfish to a small (1.64°) and large (49°) stimulus superimposed on different luminances of tungsten-light background were measured. Figure 2a shows that backgrounds have the usual desensitising effects: threshold rises at an increasing rate until it changes in proportion to background luminance (Weber's Law) as shown by the straight lines. At a background luminance of ~1 mL however, this linear relationship is interrupted by what seems to be a rod-cone break. A previous psychophysical study on goldfish found a similar break at about the same adaptation level<sup>6</sup>.

Given that straight lines of slope 2 and 0 describe the threshold-diameter data (Fig. 1), CD can be estimated from the intersection of two such lines drawn through the 1.64° and 49° stimuli respectively. Thus CDs at different background luminances (Fig. 2b) were derived from the corresponding pairs of thresholds in Fig. 2a. CD in these two goldfish fell from a dark-adapted value of 25° to about 5° in a fairly continuous fashion, remaining essentially constant above  $3 \times 10^{-3}$  mL. In humans, however, CD falls with increasing background luminance in

two distinct phases: first to an intermediate level while the rods determine threshold, and then to a lower level when the cones take over<sup>7</sup>. The fact that the goldfish CD falls without interruption, in spite of the break in the threshold-background luminance function, suggests that rod and cone signals are pooled over similar retinal areas. An equality of spatial summation in the rod and cone systems of goldfish, and the lack of it in humans can possibly be explained by the retinal organisation of the two species. In mammals, the bipolar cells contacted by rods are different from those contacted by cones<sup>8</sup>, but in cyprinid fishes, several types of bipolar cell are contacted by both rods and cones<sup>9,10</sup>. Furthermore, these fish bipolar cells receive their rod and cone input from the same retinal area, presumably leading to equal rod and cone summation areas at the bipolar and subsequent levels of processing.

**Fig. 2** *a*, Threshold stimulus radiance as a function of background luminance for 533-nm test disks of 1.64° and 49° diameter.  $\Delta$ , And  $\square$ , are data points from two goldfish of 12 cm standard length. The straight lines show Weber's Law and the dashed curves represent probable cone thresholds; *b*, relationship between critical diameter and background luminance. Each critical diameter (CD) in degrees of visual angle is found from the corresponding pair of thresholds ( $I_{49}$  and  $I_{1.64}$ ) in *a* from  $CD = 1.64(I_{49}/I_{1.64})^{1/2}$ .



It is unlikely that the spatial summation seen behaviourally in the goldfish could take place much before the retinal ganglion cells. As yet there is little physiological evidence for inter-receptor coupling in fishes as a basis for summation<sup>11</sup>, although contacts between receptors have been found<sup>10,12</sup>. Anatomical and electrophysiological studies in cyprinid fishes show that the receptive fields of bipolar cells are smaller than the smallest behavioural critical diameters, being 2.5° in diameter at most<sup>9,10,13</sup>, whereas the receptive fields of the laterally spreading elements, the horizontal and amacrine cells, are too large to account for the relatively small, light-adapted critical diameter<sup>14,15</sup>. Receptive field centres of ganglion cells are, however, found in a range of sizes that matches quite closely the range of critical diameters encountered at different adaptation levels. For the size of eye used in these behavioural experiments, the largest receptive field centres found by recording from retina or optic nerve would subtend about 30° of visual angle<sup>16,17</sup>, although fields as large or larger that may belong to ganglion cells, are also found in the optic tectum<sup>18,19</sup>. When ganglion cells are recorded in the optic nerve or tectum with the fish's eye under water, the diameters of the smallest field centres are found by different workers to be between 3° and 5° (refs 19–22).

The correspondence between the range of diameters of receptive field centres and critical diameters suggests not only a summing role for ganglion cells, but also that critical diameters in the light and dark are determined respectively by the smallest and largest field centres. To explain this, first assume that the fish responds when one or more ganglion cells signal a significant change in light stimulation. In the fully dark-adapted eye, the largest fields should be the most sensitive for large test stimuli, since they are most likely to catch the minimum number of quanta s<sup>-1</sup> required for detection. Critical diameter will then be set by the size of these fields. As background luminance is increased, the largest fields will catch more quanta from the background and will, as a result, be more desensitised than smaller fields. This phenomenon has been seen in cat ganglion cells<sup>23</sup>. Critical diameter will then be determined by progressively smaller field centres until, at a background luminance of about  $3 \times 10^{-3}$  mL and above, the smallest fields (3–5° in diameter) will determine the critical diameter. Such a mechanism may be the basis for the recruitment of smaller summing units proposed to account for the improvement of acuity with increasing luminance<sup>1,23</sup>.

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## Stability of Met-enkephalin content in brain structures of morphine-dependent or foot shock-stressed rats

THE discovery of enkephalin has suggested one more theory to explain the mechanisms of opiate tolerance and addiction. Kosterlitz and Hughes<sup>1</sup> proposed that a reduced enkephalin release might explain tolerance and physical dependence to opiates. Simantov and Snyder<sup>2</sup> supported this view by showing that the content of enkephalin was changed in brain of morphine-dependent rats. Others<sup>3-5</sup> have reported that Met-enkephalin is rapidly metabolised by brain tissue. Because of this *in vivo* lability of Met-enkephalin, particular precaution must be taken in the preparation of brain tissue samples for enkephalin assay. In fact, we have shown that by killing the rats with a microwave beam focused to the head and by taking particular precautions in preparing the brain samples we could measure Met-enkephalin concentrations which were higher (by several orders of magnitude) than the concentrations measured by other authors who failed to take such precautions<sup>6</sup>. Since the changes in the brain enkephalin content reported to occur either after a stress induced by foot shock<sup>7</sup> or during morphine dependence<sup>2</sup>, were measured by a binding assay which lacks specificity for enkephalin, we decided to repeat these experiments with a technique that specifically measures Met-enkephalin<sup>6</sup> to decide whether the brain enkephalin content changes during stress<sup>7</sup> or opiate dependence<sup>2</sup>.

We duplicated the technique described by Simantov and Snyder<sup>2</sup> to cause morphine dependence and withdrawal, the only deviation from their experimental design being the use of naltrexone 15  $\mu\text{mol kg}^{-1}$  instead of naloxone 1  $\text{mg kg}^{-1}$  to elicit morphine withdrawal.

To measure the Met-enkephalin content, male, Sprague-Dawley rats were killed with focused microwave irradiation<sup>6</sup> and the striatum and hypothalamus were dissected as outlined by Glowinski and Iversen<sup>8</sup>. The brain regions were extracted with 0.1 M acetic acid and the extracts neutralised with 1 M NaOH. The assay was carried out in polypropylene tubes. Non-labelled peptide or brain extracts were incubated with antiserum and <sup>3</sup>H-enkephalin in 0.5 ml, 0.2 M Tris buffer, pH 7.4, containing 0.1% albumin and 0.06% dextran. The <sup>3</sup>H-enkephalin bound to antibody was separated from the free <sup>3</sup>H-enkephalin by 0.2 ml 1.5% charcoal slurry containing 0.15% dextran and 0.9% NaCl. The supernatant was counted in a Beckman LS 250 liquid scintillation spectrometer. The method of preparation and the specificity of our antibody was as described previously<sup>6</sup>.

**Table 1** Morphine dependence and withdrawal on the content of Met-enkephalin in various brain structures

| Treatment                | Met-enkephalin (ng mg <sup>-1</sup> protein) |          |                     |
|--------------------------|----------------------------------------------|----------|---------------------|
|                          | Hypothalamus                                 | Striatum | Remainder of brain§ |
| Control                  | 2.6±0.9                                      | 5.0±1.0  | 1.0±0.14            |
| Morphine*                | 3.1±1.5                                      | 6.2±2.9  | 0.91±0.20           |
| Naltrexone†              | 3.8±0.9                                      | 6.3±1.1  | 0.93±0.15           |
| Morphine‡ and naltrexone | 2.8±0.9                                      | 7.0±1.5  | 0.78±0.14           |

Each value refers to the mean ± s.e.m. of the average of five determinations. The values were not corrected for recovery.

\*One morphine pellet prepared according to Gibson and Tingstad<sup>10</sup> was implanted. After 2 d, two more pellets (75 mg each) were implanted together. Rats were killed with microwave radiation 5 d after implantation of the first pellet.

†Naive rats were injected intraperitoneally with naltrexone 15  $\mu\text{mol kg}^{-1}$  and killed with microwave irradiation 1 h later.

‡Rats were treated as in \*, with the exception that naltrexone 15  $\mu\text{mol kg}^{-1}$  was injected 1 h before microwave irradiation and brain extraction.

§The remainder of brain did not contain cerebellum.

As shown in Table 1, naltrexone, implantation of morphine pellets and the combination of the two treatments, failed to change the enkephalin content of various brain regions. The data reported in Table 2 show that after 60 min of intermittent inescapable foot shock administered exactly as described by Akil *et al.*<sup>7</sup> and Madden *et al.*<sup>11</sup>, there was no change in the Met-enkephalin content of striatum, hypothalamus or the remainder of brain tissue.

**Table 2** Inescapable foot shock on content of Met-enkephalin in various structures

| Treatment   | Met-enkephalin (ng mg <sup>-1</sup> protein) |              |                    |
|-------------|----------------------------------------------|--------------|--------------------|
|             | Striatum                                     | Hypothalamus | Remainder of brain |
| Control     | 8.8±0.6                                      | 3.6±0.7      | 0.43±0.09          |
| Foot shock* | 8.1±0.3                                      | 2.8±0.3      | 0.44±0.03          |

Each value refers to the mean ± s.e.m. of an average of six determinations.

\*Foot shock consisted of 60 min intermittent inescapable foot shock (3 mA, 1 s duration, 1 per 5 s). Rats were killed with the microwave radiation immediately after shock.

Our data do not negate the possibility that in conditions of opiate dependence and withdrawal or after unavoidable foot shock, there is change in the concentration of an opiate receptor agonist of the endorphin type. Since our method measures specifically Met-enkephalin, our results merely negate that the brain content of this peptide changes during dependence. It was suggested that this increase in enkephalin was a biochemical reflection of the inactivity of enkephalinergic neurones<sup>2</sup>. It was argued that these neurones had ceased firing when the opiate receptor was occupied by saturating concentrations of morphine. We do not oppose the view that a feedback message links the occupancy of opiate receptors with the rate of firing of enkephalinergic neurones. Our results just indicate that the biochemical signal associated with the cessation of activities in enkephalinergic neurones is not an increase in brain enkephalin content. Perhaps changes in the synthesis rates of the enkephalin have a regulatory role for enkephalin steady state when neuronal activity changes. This possibility is in agreement with the finding that following the injection of naltrexone, the abrupt cessation of opiate receptor occupancy by morphine is not associated with a change in the steady state of Met-enkephalin. In contrast with reports by Akil *et al.*<sup>7</sup> and Madden *et al.*<sup>11</sup>, we failed to find an increase in enkephalin content after stress elicited with considerable foot shock. This failure may be due to the more precise methodology that is now possible in comparison with early methods<sup>2,7</sup> or could be due to the fact that the method used by Simantov and Snyder<sup>2</sup> and Akil *et al.*<sup>7</sup> also measured  $\beta$ -endorphin which, during stress, may be mobilised from pituitary and accumulates in brain tissue. Since our method is specific for Met-enkephalin, we failed to detect a possible increase in brain  $\beta$ -endorphin content.

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## Comparison of specificity of agonist and antagonist radioligand binding to $\beta$ adrenergic receptors

DIRECT radioligand binding methods have been used extensively to identify and characterise a wide range of hormone, drug and neurotransmitter receptors. Among the most intensively studied have been the  $\beta$  adrenergic receptors which mediate stimulation of the enzyme adenylate cyclase by catecholamines<sup>1</sup>. Until recently all of the successful binding studies of  $\beta$  adrenergic receptors have been performed with specific high affinity  $\beta$  adrenergic antagonists such as (–) <sup>3</sup>H-dihydroalprenolol<sup>2</sup>, (±) <sup>3</sup>H-propranolol<sup>3</sup> or (±) <sup>125</sup>I-hydroxybenzylpindolol<sup>4</sup>. Earlier attempts to use radioligand  $\beta$  adrenergic agonists were generally frustrated by a great deal of nonspecific (that is non-receptor) binding<sup>5</sup>. We have developed a high affinity radiolabelled  $\beta$  adrenergic agonist, <sup>3</sup>H-hydroxybenzylisoprenaline, which, when used in conjunction with high concentrations of catechol and ascorbic acid which block non-receptor binding sites, seems to be a useful ligand for studying the  $\beta$ -adrenergic receptors<sup>6</sup>. We present here the results of parallel studies of agonist (<sup>3</sup>H-hydroxybenzylisoprenaline, HBI) and antagonist (<sup>3</sup>H-dihydroalprenolol, DHA) binding to  $\beta$ -adrenergic receptors in frog erythrocyte membranes. The ability of sixteen  $\beta$ -adrenergic agents (agonists and antagonists) to competitively inhibit binding of the two ligands in identical assay conditions (including catechol and ascorbate) has been compared. The results suggest an impressive coincidence of the binding data obtained with the two radioligands. These results are in contrast with those recently described for several central nervous system neurotransmitter receptors where it seems to be possible to identify discrete 'agonist' and 'antagonist' conformations of the receptors by performing binding studies with either a radiolabelled agonist or antagonist respectively<sup>7-11</sup>.

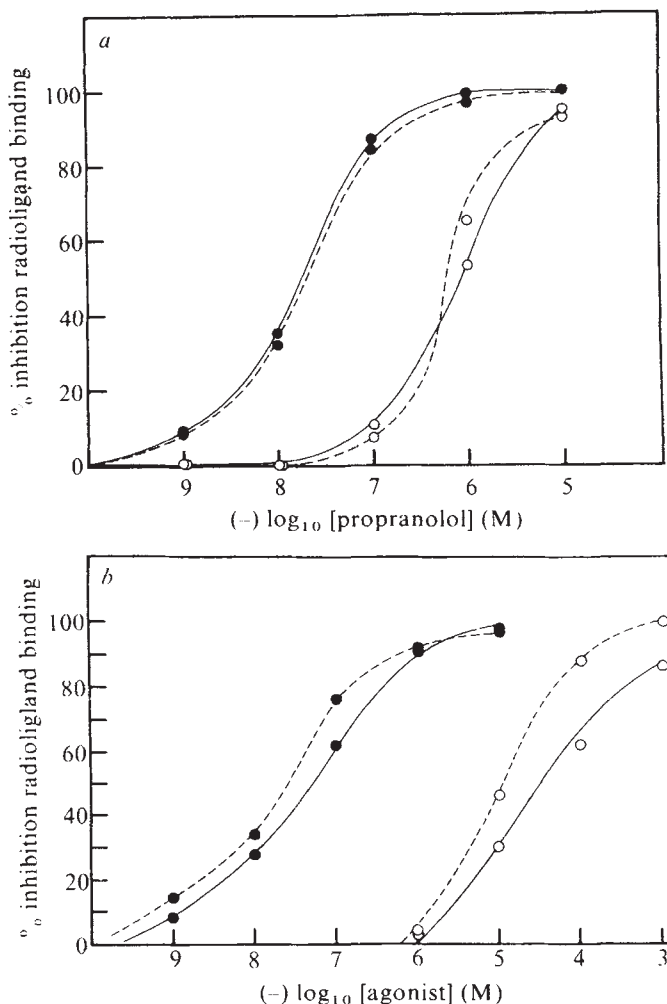
The sources of all materials used have been reported previously<sup>6,12</sup>. The frog erythrocyte membrane preparation used in these studies was that previously described<sup>13</sup> and was obtained by centrifuging crude frog erythrocyte lysates over a cushion of 50% sucrose at 2,000g for 10 min. The membranous material which does not sediment through the sucrose was collected and used in all binding studies. These preparations have a five-to tenfold enhancement of  $\beta$ -receptor binding and adenylate cyclase activity as compared with crude lysates<sup>13</sup>. Binding assays with both radioligands were carried out essentially as before<sup>6,14</sup>.

EC<sub>50</sub> values (concentrations of agents causing 50% displacement of specific binding) for any agent were determined in the same experiment using both radioligands. Five concentrations of each agent were generally tested. We have previously demonstrated that binding of both radioligands reaches steady state within the 10 min incubations at 37 °C (refs 6, 15). Dissociation constants ( $K_D$ ) of various competing agents were not calculated since such calculations require an estimate of the  $K_D$  of the radioligands. We have previously demonstrated that <sup>3</sup>H-HBI binding is only slowly reversible and hence Scatchard<sup>16</sup> and similar analyses do not provide meaningful estimates of its affinity for the  $\beta$  receptors.

The ability of the (–) and (+) stereoisomers of propranolol to inhibit the binding of the two radioligands to frog erythrocyte membranes is depicted in Fig. 1a. It can be observed that the two sets of curves are virtually superimposable. The result is typical of the displacement curves obtained when  $\beta$ -adrenergic antagonists were used to inhibit radioligand binding.

The ability of  $\beta$ -adrenergic agonists to inhibit the binding of the two radioligands is depicted in Fig. 1b. These results are

typical of the displacement curves obtained with agonists. For potent agonists, such as (±)HBI, the displacement curve for (±)<sup>3</sup>H-HBI was usually slightly (one-to twofold) to the left of that for (–)<sup>3</sup>H-DHA. For weak agonists, however, such as (+)adrenaline, the curve for inhibition of (±)<sup>3</sup>H-HBI was displaced somewhat more to the left of that for (–)<sup>3</sup>H-DHA (in this case approximately threefold).



**Fig. 1** *a*, Inhibition of radioligand binding to frog erythrocyte membrane  $\beta$ -adrenergic receptors by stereoisomers of propranolol. Membranes were incubated in a final assay volume of 150  $\mu$ l which contained Tris-HCl, 50 mM (pH 7.5), 15 mM MgCl<sub>2</sub> and 40,000–50,000 c.p.m. (10 nM) (±)<sup>3</sup>H-HBI (broken line) or ~60,000 c.p.m. (~10 nM) (–)<sup>3</sup>H-DHA (solid line) in the presence or absence of various concentrations of adrenergic agents (–) propranolol (●) and (+) propranolol (○). All assays with either radioligand also contained catechol  $3 \times 10^{-4}$  M and ascorbic acid,  $8 \times 10^{-4}$  M to block non-receptor binding sites which might bind the labelled agonist. All incubations were performed for 10 min at 37 °C and were terminated by vacuum filtration through a Whatman GFC glass fibre filter followed by washing with either 10 ml (<sup>3</sup>H-DHA) or 20 ml (<sup>3</sup>H-HBI) of ice cold incubation buffer. Filters were dried overnight then counted in a triton-toluene based fluor in a Packard liquid scintillation spectrometer at an efficiency of 40%. Separate incubations were also carried out in the presence of  $10^{-5}$  M (±)propranolol to determine 'nonspecific' binding. Specific or  $\beta$ -receptor binding in all cases was taken as total minus nonspecific binding. In separate experiments we demonstrated that these washing procedures reduced nonspecific binding while having no effect on specific binding<sup>6,12</sup>. At the radioligand concentrations used in these studies 'specific' binding was routinely ~70% of total binding with (±)<sup>3</sup>H-HBI and ~95% of total binding with (–)<sup>3</sup>H-DHA. Results shown are the means of three experiments. *b*, Inhibition of radioligand binding to frog erythrocyte membrane  $\beta$ -adrenergic receptors by a potent and a weak agonist. Results shown are the means of two or three experiments. Broken line, (±)<sup>3</sup>H-HBI; solid line, (–)<sup>3</sup>H-DHA. ●, (±)HBI; ○, (+)adrenaline.



A summary of  $EC_{50}$  values for the 16 adrenergic agonists used in this study as inhibitors of  $^3H$ -HBI and  $^3H$ -DHA binding is presented in Fig. 2 and Table 1. As shown in Fig. 2, the correlation between the two sets of data is excellent. When  $EC_{50}$  values for all antagonists for  $^3H$ -HBI binding were compared with those for  $^3H$ -DHA binding no statistically significant difference was found (paired  $t$  test,  $P > 0.05$ ).

It may be noted that some of the  $EC_{50}$  values obtained in the present studies, especially those for catecholamine agonists are somewhat lower than values obtained previously<sup>12</sup>. This is apparently due to the protective effect of the catechol and ascorbate in the incubations on these agents and underscores the importance of comparing  $^3H$ -HBI and  $^3H$ -DHA  $EC_{50}$  values in identical experimental conditions.

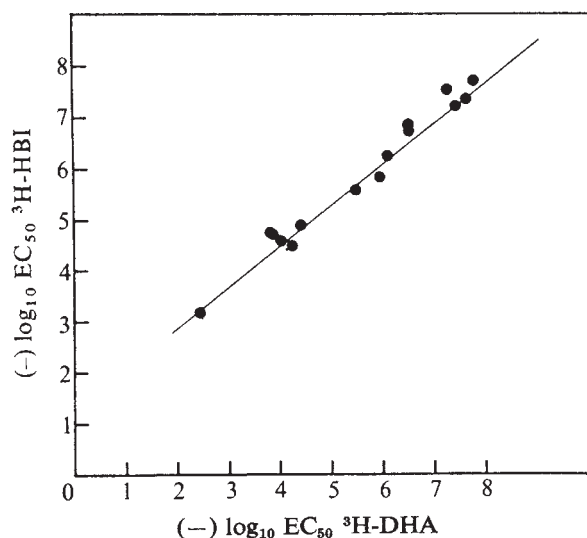


Fig. 2 Correlation of  $EC_{50}$  values for  $\beta$ -adrenergic agents determined by inhibition of  $(-)^3H$ -DHA and  $(\pm)^3H$ -HBI binding to frog erythrocyte membranes. The line was determined by linear regression analysis.  $r = 0.99$ .

The widespread distribution of  $\beta$ -adrenergic receptors in mammalian and non-mammalian species, their close relationship with the enzyme adenylate cyclase and the availability of several radiolabelled  $\beta$ -adrenergic antagonist ligands has served to focus a great deal of experimental effort on the  $\beta$ -adrenergic receptors in the past several years<sup>15</sup>. In a number of respects these receptors seem to serve as a prototype of adenylate cyclase-coupled receptors. Unlike the situation with receptors for several polypeptide hormones, there are a wide variety of both agonist and antagonist agents whose interactions with the  $\beta$  receptors can be studied. Until recently, however, only  $\beta$ -adrenergic antagonist radioligands had been successfully used for direct binding studies of these receptors. With the development of  $(\pm)^3H$ -HBI (ref. 6) it has become possible, for the first time, to perform parallel studies of an adenylate cyclase-coupled receptor with both a radiolabelled agonist and antagonist. As noted above, such studies are of particular interest in view of recent reports that for several central nervous system neurotransmitter receptors, discrete 'agonist' and 'antagonist' states of the receptors can be identified with such radioligands<sup>7-11</sup>. In such studies it has generally been found that agonists have considerably higher affinity (one to three orders of magnitude) for the sites labelled with the radiolabelled agonist, whereas antagonists have much higher affinity for sites labelled with radiolabelled antagonists.

The data presented here apparently indicate a somewhat different situation for the adenylate cyclase-coupled  $\beta$ -adrenergic

Table 1  $EC_{50}$  values for  $\beta$ -adrenergic agents determined by inhibition of  $(-)^3H$ -dihydroalprenolol and  $(\pm)^3H$ -hydroxybenzylisoprenaline binding to frog erythrocyte membranes

| Agent                                        | n | EC <sub>50</sub> (nM)        |                              |
|----------------------------------------------|---|------------------------------|------------------------------|
| Antagonists                                  |   |                              |                              |
| (-) <sup>3</sup> H-Propranolol               | 3 | (-) <sup>3</sup> H-DHA 0.018 | (±) <sup>3</sup> H-HBI 0.020 |
| (+) <sup>3</sup> H-Propranolol               | 3 | 0.800                        | 0.600                        |
| (-) <sup>3</sup> H-Alprenolol                | 3 | 0.025                        | 0.045                        |
| (±) <sup>3</sup> H-Dichlorisoprenaline       | 3 | 1.2                          | 1.5                          |
| (±) <sup>3</sup> H-Phenylephrine             | 2 | 60                           | 35                           |
| Dopamine                                     | 2 | 4,000                        | 700                          |
| Agonists and partial agonists                |   |                              |                              |
| (±) <sup>3</sup> H-Hydroxybenzylisoprenaline | 3 | 0.060                        | 0.030                        |
| (±) <sup>3</sup> H-MJ 9184-1                 | 2 | 0.044                        | 0.060                        |
| (-) <sup>3</sup> H-Isoprenaline              | 3 | 0.340                        | 0.180                        |
| (-) <sup>3</sup> H-Soterenol                 | 3 | 0.320                        | 0.150                        |
| (-) <sup>3</sup> H-Adrenaline                | 2 | 3.5                          | 2.5                          |
| (-) <sup>3</sup> H-Noradrenaline             | 2 | 100                          | 28                           |
| (+) <sup>3</sup> H-Isoprenaline              | 2 | 150                          | 20                           |
| (+) <sup>3</sup> H-Adrenaline                | 2 | 42                           | 13                           |
| (+) <sup>3</sup> H-Noradrenaline             | 2 | > 1,000                      | 380                          |
| (±) <sup>3</sup> H-Cobefrin                  | 3 | 150                          | 18                           |

n, Number of experiments determined in duplicate. Inhibition curves were plotted as shown in Fig. 1a and b for all agents for the determination of  $EC_{50}$  values.

receptors of frog erythrocytes. Binding displacement curves using the radioligand agonist and antagonist seem to be in close agreement. A slight but systematic and statistically significant difference does exist for weak agonists which are somewhat (two-to eightfold) more potent in displacing the labelled agonist than the antagonist. The significance of this finding is not clear at present.

These findings do not exclude the possibility that there may be separate 'agonist' and 'antagonist' conformations of the  $\beta$ -adrenergic receptors in frog erythrocyte membranes. If such do exist, however, our data indicate that they are freely interconvertible in the membranes and in the assay conditions used in these experiments. These observations are in marked contrast to several other receptor systems noted above, wherein agonist and antagonist binding give strikingly different results<sup>7-11</sup>.

The ability to study the adenylate cyclase-coupled  $\beta$ -adrenergic receptors with both agonist and antagonist radioligands should provide new insights into the nature and regulation of these receptors.

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## Development of $\beta$ -adrenergic receptor subsensitivity by antidepressants

CONSIDERABLE evidence exists that the tricyclic antidepressant drugs are selective inhibitors of monoamine uptake into monoaminergic nerve terminals<sup>1,2</sup>. These drugs also potentiate pharmacological responses to the monoamines in both the peripheral and central nervous systems<sup>3-6</sup>. These effects do not explain the discrepancy between the time-course of the biochemical and pharmacological responses which are elicited by these antidepressant drugs within minutes or hours and their clinical antidepressant action which requires treatment for weeks for efficacy to be reached. Furthermore, desipramine, an antidepressant drug, can potentiate neuronal responses to noradrenaline and dopamine in the caudate nucleus<sup>7</sup>, although it does not block the catecholamine uptake in this area of the brain<sup>1,2</sup>. Finally the antidepressant iprindole does not influence noradrenaline turnover<sup>8</sup> or metabolism<sup>9,10</sup> or its uptake into catecholaminergic neurones<sup>11</sup>, but is effective in potentiating the responses of single cortical and caudate neurones to monoamines<sup>12</sup>. Vetulani and his associates<sup>13</sup> suggested that the therapeutic action of tricyclic antidepressants may be related to postsynaptic adaptive changes in the sensitivity of the noradrenergic adenylate cyclase receptor system rather than to acute presynaptic events. The purpose of the experiments described here was to explore the molecular basis for antidepressant-induced noradrenergic subsensitivity by examining the kinetic properties of the  $\beta$ -adrenergic receptors in the microsomal suspension of rat brain by using a potent  $\beta$ -adrenergic receptor antagonist <sup>3</sup>H-dihydroalprenolol as a radiolabelled ligand<sup>14-19</sup>. Our results indicate that the main mechanism is a reduction in the number of receptors.

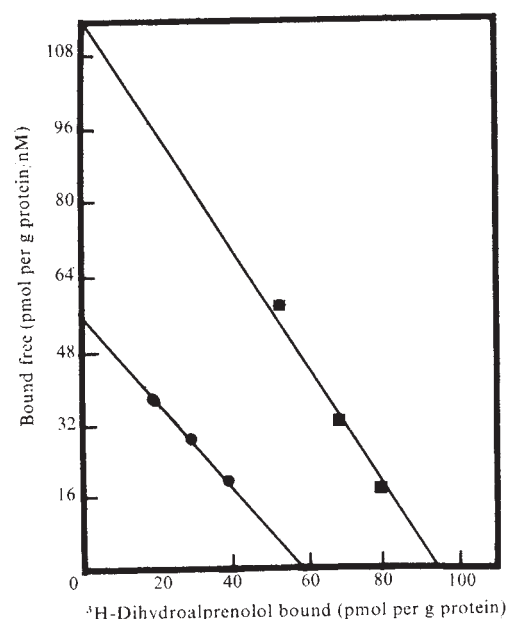
Specific <sup>3</sup>H-dihydroalprenolol binding to the microsomal fraction obtained from brain in control and in desipramine, iprindole- and doxepin-treated rats is shown in Table 1. When the

**Table 1** Effect of chronic administration of antidepressants on specific dihydroalprenolol binding

| Drug treatment | Specific <sup>3</sup> H-dihydroalprenolol binding pmol per g protein |
|----------------|----------------------------------------------------------------------|
| None           | 70.0 $\pm$ 3.5                                                       |
| Desipramine    | 24.2 $\pm$ 1.5<br>( <i>P</i> < 0.001)                                |
| Iprindole      | 40.4 $\pm$ 2.8<br>( <i>P</i> < 0.001)                                |
| Doxepin        | 48.6 $\pm$ 2.4<br>( <i>P</i> < 0.001)                                |

Desipramine, iprindole and doxepin were injected intraperitoneally in male, Sprague-Dawley rats, weighing 180–220 g, at a dose of 10 mg per kg body weight daily for 6 weeks. The rats were killed 1 h after the last injection by decapitation and the brains were rapidly removed and chilled in ice-cold 0.9% NaCl. The whole brain minus cerebellum was homogenised using a Brinkman Polytron, in 20 volumes of ice-cold 0.05 M Tris buffer (pH 8.0 at 25°), and centrifuged at 49,000*g* for 15 min. The pellet was rehomogenised in the same buffer and centrifuged as before. The pellet was finally resuspended in 50 volumes of Tris buffer (pH 8.0 at 25°) and used fresh for the binding assay. <sup>3</sup>H-dihydroalprenolol (32 Ci mmol<sup>-1</sup>) binding was determined at 23 °C by combining 0.97 ml of the above particulate suspension, 4.0 nM <sup>3</sup>H-dihydroalprenolol and sufficient distilled water to make 1 ml as the final volume of the reaction mixture. After 20 min incubation, the reaction mixtures were filtered under reduced pressure through Whatman glass fibres (GF/B). The filters were rinsed four times with 4 ml ice-cold Tris buffer to remove most of the unbound radioactive ligand. The filter papers were dried and placed in a counting vial containing 10 ml of Scintiverse (Fisher) and counted in a Packard Tri-carb liquid scintillation spectrometer (Model 3380) at 30% efficiency. Corrections were made for nonspecific accumulation of radioactivity by assaying parallel incubations which contained a large excess (100  $\mu$ M) of noradrenaline. Specific binding, defined as the difference between total and nonspecific radioactivity was 60–70% of the total binding. Values are means  $\pm$  s.e.m.

concentration of <sup>3</sup>H-dihydroalprenolol was 4 nM, the specific binding to the control preparation was found to be 70 pmol per g protein. Chronic treatment of rats with desipramine, iprindole and doxepin decreased the specific binding by 65.5%, 42.3% and 30.6% as compared to control, respectively. This observation suggests that the resistance of adenylate cyclase to noradrenaline following chronic administration of antidepressants is due to subsensitivity of noradrenergic  $\beta$ -receptors. This decreased sensitivity of  $\beta$ -adrenergic receptors towards catecholamines following chronic administration of antidepressants may be due to alterations of the apparent affinity of these receptors to dihydroalprenolol or to a decrease in the number of  $\beta$ -adrenergic receptor sites. The dissociation constants of <sup>3</sup>H-dihydroalprenolol and the densities of the  $\beta$ -adrenergic receptors in control and desipramine-treated rat brain membrane fractions were determined by measuring the specific binding of various concentrations of <sup>3</sup>H-dihydroalprenolol and analysing the data by the method of Scatchard<sup>20</sup>. Only a single class of high affinity binding sites (Fig. 1) for <sup>3</sup>H-dihydroalprenolol could be detected in rat brain microsomes.



**Fig. 1** Scatchard analysis of specific <sup>3</sup>H-dihydroalprenolol binding in brain microsomal fractions of control (■) and desipramine (●)-treated rats. The concentrations of <sup>3</sup>H-dihydroalprenolol used varied from 0.5 to 4.0 nM. (Procedure as for Table 1.) Dissociation constant and maximal number of binding sites were calculated from this figure and given in Table 2.

This observation is in agreement with the previously reported results of Bylund and Snyder<sup>19</sup>. The dissociation of <sup>3</sup>H-dihydroalprenolol binding to control rat brain particulate fraction was found to be 0.82 nM and this value is similar to the affinity constant (1.3 nM) of  $\beta$ -adrenergic receptors to <sup>3</sup>H-dihydroalprenolol reported by Bylund and Snyder<sup>19</sup>. Although the dissociation constant of <sup>3</sup>H-dihydroalprenolol to  $\beta$ -adrenergic receptors did not increase significantly, the density of receptors markedly decreased following chronic administration of desipramine (Table 2). This would suggest that chronic administration of desipramine leads to a decrease in the density of  $\beta$ -adrenergic receptors.

There are several lines of evidence indicating that modification of the presynaptic activity of noradrenaline, by altering, its rate of synthesis, release, uptake or storage, causes changes at the postsynaptic sites which are manifested as supersensitivity or subsensitivity. Such alterations in the sensitivity of the postsynaptic receptors for noradrenaline have been demonstrated by measuring noradrenaline-stimulated accumu-



**Table 2** Scatchard analysis of specific  $^3\text{H}$ -dihydroalprenolol binding to brains of control and chronically desipramine-treated rats

| Brain preparation   | Dissociation constant (nM) | Density of receptors (pmol per g protein) |
|---------------------|----------------------------|-------------------------------------------|
| Control             | $0.82 \pm 0.08$            | $95 \pm 5$                                |
| Desipramine treated | $1.09 \pm 0.14$            | $59 \pm 3$                                |
|                     | ( $P > 0.05$ )             | ( $P < 0.001$ )                           |

The dissociation constants of  $^3\text{H}$ -dihydroalprenolol and the density of binding sites were determined by Scatchard analysis<sup>20</sup>. The procedures for the preparation of the particulate fraction and measurement of specific binding of  $^3\text{H}$ -dihydroalprenolol to membrane fractions are described in Table 1. Each experimental point is the average of nine determinations obtained from three animals in each group.

lation of cyclic AMP after administration of drugs such as 6-hydroxydopamine, cocaine and reserpine<sup>21-23</sup>. In general, drugs that decrease the activity of noradrenaline at the pre-synaptic sites cause supersensitivity as opposed to drugs that produce subsensitivity by increasing noradrenaline levels at the synaptic cleft. Vetulani and his associates<sup>13</sup> have provided evidence that chronic administration of antidepressants leads to subsensitivity of noradrenaline stimulated adenylate cyclase. Our results suggest that this resistant state of adenylate cyclase towards noradrenaline after chronic administration of antidepressants is due to a decrease in the number of  $\beta$ -adrenergic receptors. A hypothesis that  $\beta$ -adrenergic catecholamines are able to regulate catecholamine sensitivity of tissues *in vivo* by regulating the number of  $\beta$ -adrenergic receptors has been advanced<sup>24</sup>. The effect of desipramine provides some support to the above hypothesis. On the other hand, iprindole, with a minimal effect at presynaptic neurones, caused  $\beta$ -adrenergic receptor subsensitivity (Table 1).

The results suggests that although the level of noradrenaline may have an important role in controlling  $\beta$ -adrenergic receptor density, the concentration of this neurotransmitter at the synaptic cleft is not the only mechanism for the regulation of  $\beta$ -adrenergic receptor concentration.

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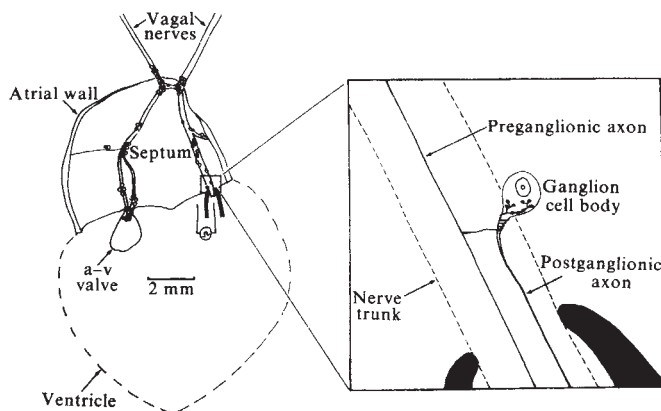
## Formation of synapses between parasympathetic neurones deprived of preganglionic innervation

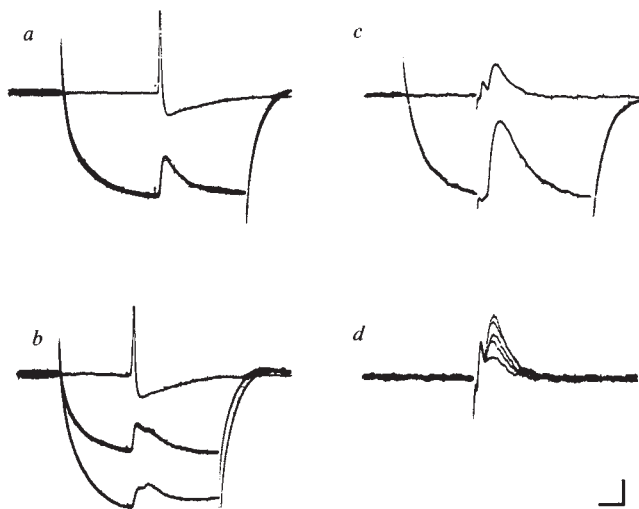
MUSCLE fibres will accept innervation from a foreign nerve only if synaptic transmission from the appropriate nerve has been interrupted<sup>1</sup>. The native nerve supply thus exerts an influence which renders muscle resistant to abnormal innervation. Do such influences act to prevent neurones from receiving inappropriate synapses? In the central nervous system (CNS) surviving inputs to partially denervated neurones will sprout to form additional synapses on those same neurones<sup>2-4</sup>. Furthermore, in partially denervated autonomic ganglia, surviving inputs will sprout to form synapses on postganglionic neurones they did not innervate originally<sup>5-7</sup>. But there has been no electrophysiological demonstration that denervated neurones will spontaneously receive synapses from a class of nerve cells with which there is normally no contact. We report here that the parasympathetic ganglion cells of the frog heart form functional synaptic connections with each other when their preganglionic synaptic input is removed; these intraganglionic connections do not occur normally.

The parasympathetic ganglion cells of the frog heart lie along two major nerve trunks in the interatrial septum (Fig. 1a)<sup>8</sup>. These trunks, which are extensions of vagal nerve branches, enter the heart at the sinus venosus, traverse the septum and pass into the ventricle. Stimulation of the nerve trunks at the point where they pass into the ventricle (Fig. 1b) results in antidromic activation of most ganglion cells in the septum (see Fig. 2a).

We searched for synaptic connections between ganglion cells by stimulating the nerve trunks antidromically and recording intracellularly from individual cells. In normal ganglia this procedure is complicated by the fact that synaptic potentials may result also from antidromic stimulation of preganglionic axons, branches of which sometimes extend as far as the ventricular edge of the septum (Fig. 1b)<sup>9</sup>. This complication was avoided by carrying out all experiments 2 d or more after vagotomy, by which time preganglionic transmission has ceased. The hearts of *Rana pipiens* were denervated by bilateral resection of the vagus nerve<sup>10</sup>, and reinnervation was delayed

**Fig. 1** Schematic view of the frog interatrial septum. *a*, Ganglion cell bodies (not drawn to scale) are seen along the two nerve trunks that pass through the septum. One of the a-v valves has been removed, and the nerve trunk has been drawn into a suction electrode (stippled) for antidromic stimulation of ganglion cells (see expanded view in *b*).





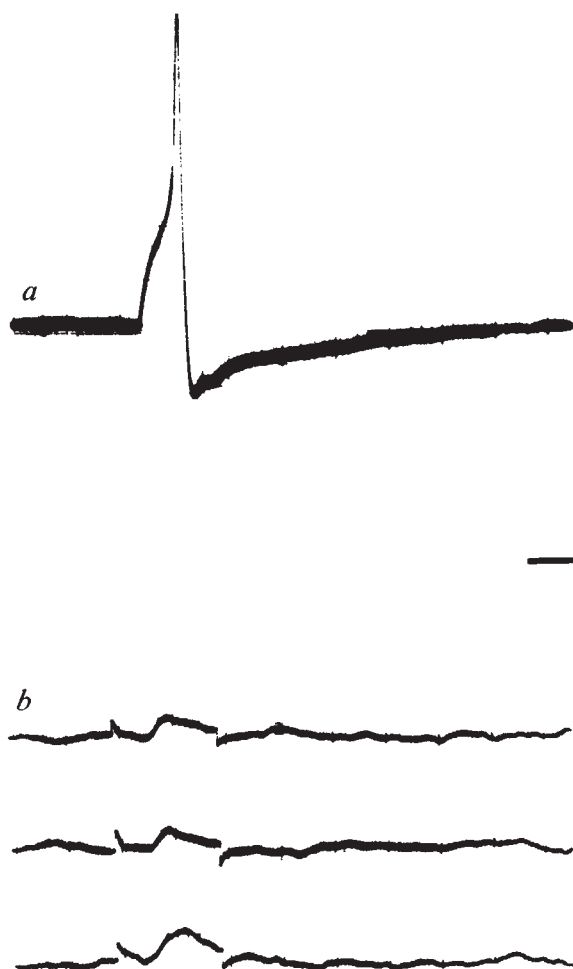
**Fig. 2** Action potentials and synaptic potentials recorded intracellularly from frog cardiac cells. *a*, An antidromic action potential was elicited by nerve trunk stimulation (upper trace). When the cell was hyperpolarised by current injected through the recording electrode, the action potential failed to invade the cell body and appeared instead as a monotonic depolarisation (lower trace). This neurone was denervated 4 d previously to allow degeneration of the preganglionic fibres. *b* And *c*, both action potentials and synaptic potentials were elicited from ganglion cells deprived of preganglionic input for 2–3 months. *b*, Neurone responding to antidromic stimulation with an action potential, apparently without a synaptic potential (upper trace). When the cell was hyperpolarised by current passed through the recording electrode, however, a small synaptic potential was seen on the falling phase of the blocked action potential (middle trace). Further hyperpolarisation reduced the amplitude of the blocked action potential and enhanced the synaptic potential (bottom trace). *c*, Antidromic stimulation elicited an action potential that did not invade the cell body and a synaptic potential. The action potential was not observed on hyperpolarisation and the synaptic potential was enhanced. *d*, The amplitude of this same synaptic potential fluctuated in response to repeated stimuli of constant intensity in a manner characteristic of low quantal content synaptic transmission (five stimuli, delivered at 0.3 Hz). Horizontal calibration 20 ms; vertical calibration, 20 mV in *a* and *b*, 10 mV in *c*, 5 mV in *d*. Experiments were carried out in Leibovitz-15 medium diluted to 40% and supplemented with inorganic salts, glucose and buffer to give the following concentrations (in mM): NaCl, 114; KCl, 2.1; CaCl<sub>2</sub>, 3.6; MgCl<sub>2</sub>, 0.8; glucose, 5; HEPES, 5; pH, 7.2. Electrophysiological recordings were made with micropipettes filled with 4 M potassium acetate and bevelled to resistances of 60–120 MΩ. Only cells which had resting potentials more negative than  $-40$  mV were studied.

by a second resection 50–60 d after the first. In experiments carried out long after denervation, the possibility of vagal reinnervation was checked by orthodromic stimulation of the vagus nerves; septa which had been reinnervated were not used.

Synaptic potentials elicited by antidromic nerve stimulation were not detected in septa denervated for short times. In nine animals examined 2–5 d after denervation, no synaptic potentials were encountered in 200 neurones examined (175 responded with antidromic action potentials). We conclude that intrinsic synaptic connections are absent in normally innervated septa. In contrast, however, in seven septa denervated for 60–100 d, antidromic stimulation elicited synaptic potentials from 52 of 107 neurones examined (93 responded with antidromic action potentials). No significant difference in resting potentials or input resistance was noted between these cells and those denervated for short times. Sample recordings from two neurones that show both antidromic action potentials and synaptic potentials are reproduced in Fig. 2*b* and *c*. The synaptic origin of these potentials is indicated by the fact that they were enhanced by hyperpolarisation, that they were abolished by  $10^{-4}$  M *d*-tubocurarine, and that their amplitudes often showed fluctuations characteristic of low quantal content transmission (Fig. 2*d*).

Two kinds of experiments were carried out to show that the synaptic potentials recorded in denervated ganglia arise from connections between the ganglion cells. In the first, to demonstrate synaptic coupling between pairs of ganglion cells, an intracellular recording was made from one neurone while the cell bodies of neighbouring neurones were stimulated extracellularly (see Fig. 3*a*). The probability of success of this experiment was low since usually less than 2% of the cells in the septum were accessible for stimulation within a single field of view (diameter 400 μm). In 2 of 388 pairs tested, extracellular stimulation of one ganglion cell produced a synaptic potential in another (Fig. 3*b*). In the second group of experiments, we searched for synaptic potentials in septa which had been denervated chronically and then isolated and maintained *in vitro*. Since parasymphathetic ganglion cells are the only neurones whose cell bodies lie within the septum, only these cells should remain viable in isolated septa. Indeed, when normal heart septa were cultured at 22 °C, preganglionic synaptic transmission failed in 24–36 h. In contrast, synaptic potentials could be elicited by antidromic nerve trunk stimulation from many neurones in septa denervated for 3 months and then maintained *in vitro* for 3–4 d (14 examples in 46 cells examined; culture medium as described in Fig. 2 legend, supplemented with foetal calf serum and antibiotics). We conclude that the synaptic potentials elicited in isolated septa result from synaptic interaction between ganglion cells.

Our results indicate that prolonged loss of normal preganglionic input leads to the formation of excitatory synapses



**Fig. 3** Direct demonstration of synaptic interaction between ganglion cells. *a*, Stimulation by a blunt extracellular micropipette elicited an action potential from a ganglion cell. *b* (a different experiment), three successive e.p.s.ps recorded from a neurone in response to extracellular stimulation of another nerve cell 300 μm distant (40-ms stimulation pulses). Calibration: 10 mV, 20 ms.



between frog parasympathetic neurones. These postganglionic collateral synapses do not exist normally in frog, whereas they do in the mudpuppy cardiac ganglion<sup>11,12</sup> and in the superior cervical ganglion of guinea pig<sup>7</sup>. These connections also develop between dissociated rat sympathetic ganglion cells in culture<sup>13,14</sup>. The capacity to form collateral synapses in frog presumably arises in part because acetylcholine is both the pre- and the postganglionic transmitter in the cardiac ganglion. Experiments are now in progress to determine the fate of these intraganglionic synapses following reinnervation of ganglion cells by their normal vagal inputs.

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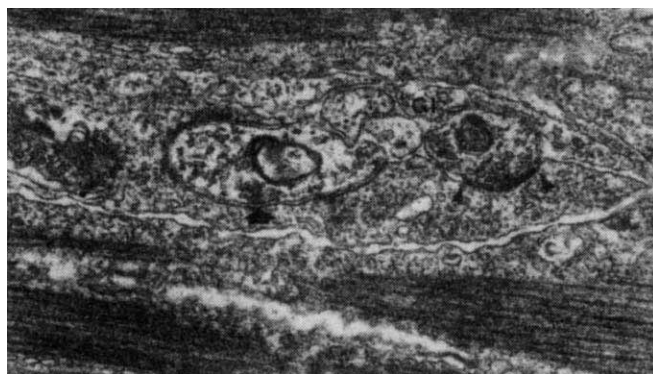
## Synaptic and septate neuromuscular junctions in embryonic lobster muscle

It is generally accepted that there is not enough genetic material to programme uniquely the structure of each neurone in multicellular animals. This then presents the problem of determining how a growing neurone finds and identifies the correct postsynaptic structures. Work on the visual system of *Daphnia* has demonstrated that the primary sensory neurones form transient gap junctions with undifferentiated neuroblasts in the optic lamina<sup>1</sup>. No similar work has been reported for interneurons or motor neurones, however. For instance, we do not know how developing motor neurones find the proper muscle in the periphery<sup>2</sup>. I studied neuromuscular development in lobster embryos and report here that motor neurones form septate junctions with developing muscle cells in the abdomen. In addition, mature neuromuscular synapses are present in the embryo as early as the third to fourth month of the total 9 month development.

Crustacean neuromuscular systems offer several advantages for studying developmental processes. Each muscle is innervated by a few identifiable neurones, at least one of which is inhibitory. Muscle fibres each receive multiple inputs, but not all fibres within a muscle are identically innervated<sup>3,4</sup>. For these reasons, it has been suggested that these systems might serve as useful models to study processes which occur in central nervous systems<sup>5</sup>. Previous work on developing arthropod neuromuscular systems has been limited primarily to studies on insect larvae<sup>6,7</sup>. One ultrastructural study on developing crayfish opener muscle suggested that active synapses were not present just before hatching<sup>8</sup>.

Egg-bearing lobsters (*Homarus americanus* Milne-Edwards) were trapped in local waters around Woods Hole, Massachusetts, and kept in tanks of running seawater at ambient temperatures. These animals extrude and fertilise the eggs during September or October, then brood them under the abdomen until the following May or June, when the larvae all hatch within 1–2 d (ref. 9). Development is not constant during this period; after extrusion, development proceeds for several months until water temperature falls below about 10 °C during December. There is little development for the following 5–6 months, but when water temperature again reaches about 10 °C, during late April, development proceeds to hatching<sup>10</sup>. The embryos used in this study were removed in early April. Abdomens were isolated from the thorax and the telson was removed to permit penetration of fixative for electron microscopy. Fixation was in 2% glutaraldehyde, 1% formaldehyde, 100 mM cacodylate at pH 7.4, 4% sucrose and 0.5% NaCl. Manganese chloride (0.1%) was added to the initial fixative to prevent release of synaptic vesicles during fixation (unpublished). Sections were cut on a diamond knife and post-stained with uranyl acetate and lead citrate.

The abdominal muscles do not differentiate synchronously during development. In all embryos fixed between December and April it was possible to observe fully differentiated short (1.5–2.5  $\mu$ m) and long (2.5–4.0  $\mu$ m) sarcomere muscle fibres as well as undifferentiated muscle cells. Synaptic profiles, which were generally similar to those seen in adult muscle, were observed only on mature muscle fibres (Fig. 1). Growing axons were characterised by the presence of two populations of vesicles, one with a diameter

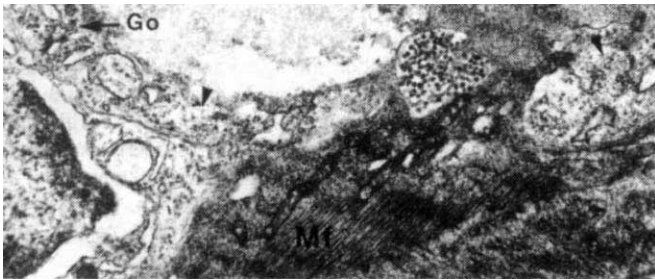


**Fig. 1** Excitatory and inhibitory neuromuscular junctions on embryonic lobster muscle. The excitatory terminal (right) has the characteristic round synaptic vesicles. The synaptic region has a heavily stained pre- and postsynaptic membrane (between arrowheads). Densely staining material is present in the synaptic cleft. The inhibitory terminal (left) contains smaller, irregular vesicles and the pre- and postsynaptic membranes are characteristically not heavily stained (see ref. 8). A presynaptic dense body, with vesicles clustered around it, is present (arrow). These nerve terminals are on a long sarcomere fibre; similar terminals are observed on short sarcomere fibres. ( $\times 28,200$ .)

of about 40–50 nm and the other a diameter of about 60–90 nm (Figs 2 and 3). The former are the same size as the vesicles normally observed in lobster excitatory synapses. But, in the growing axons many of the vesicles were electron opaque while those in neuromuscular synapses are agranular. The second, larger type of vesicle is similar to the dense-cored vesicles of unknown function which have been described in a wide variety of neuromuscular synapses<sup>11</sup>.

The growing ends of the axons are always observed in proximity to embryonic muscle cells and are characterised by several features. First, vesicles are invariably present in rather high densities. Second, growth cones or 'microspikes'<sup>14</sup> are present and are packed with vesicles (Figs 2, 3). Third, a number of tubular structures are



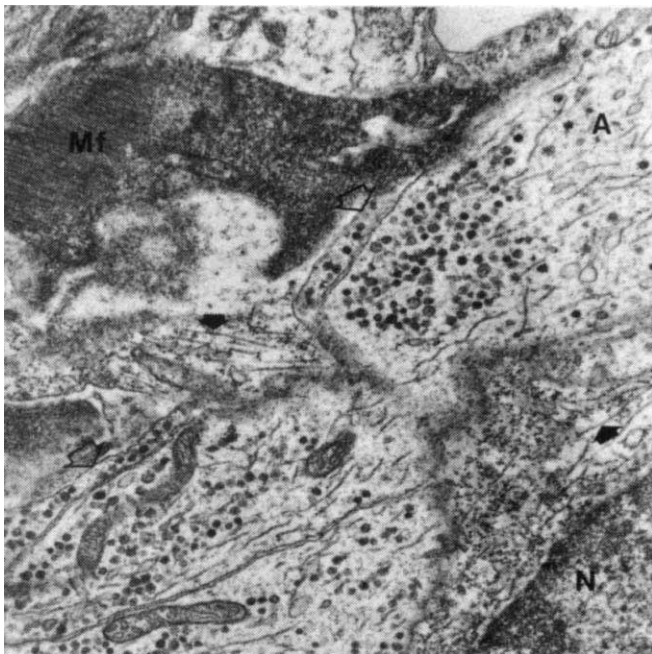


**Fig. 2** Growth cone of a motoneurone in abdominal muscle of embryonic lobster. The growth cone is characterised by the presence of numerous vesicles ranging from about 50–90 nm in diameter (see text). The cone, which is adjacent to an embryonic myotubule, appears to be in the process of dividing. Note microtubules (arrowheads), Golgi apparatus (Go) and myofibrils (Mf) in the myotube. ( $\times 9,000$ .)

present. Some of these seem to be neurotubules but others enlarge into vesicular structures and may represent some type of endoplasmic reticulum. These axons, unlike mature motor axons, are never covered with glial cells.

The muscle cells that are associated with the growing axons have a distinctive appearance different from mature muscle fibres. They resemble the myotubes described for developing vertebrate muscle<sup>12</sup>. There are never any sarcomeres present but near the border there is always a band of myosin with interdigitating actin filaments (Figs 2, 3). This band is often adjacent to an area of very dense sarcoplasm. The borders of the muscle cell always contain microtubules and a granular substance, probably glycogen. Microtubules are present in mature muscle fibres, but never in the density observed in these embryonic muscle cells, except at the tendonal ends (unpublished, and see ref. 13).

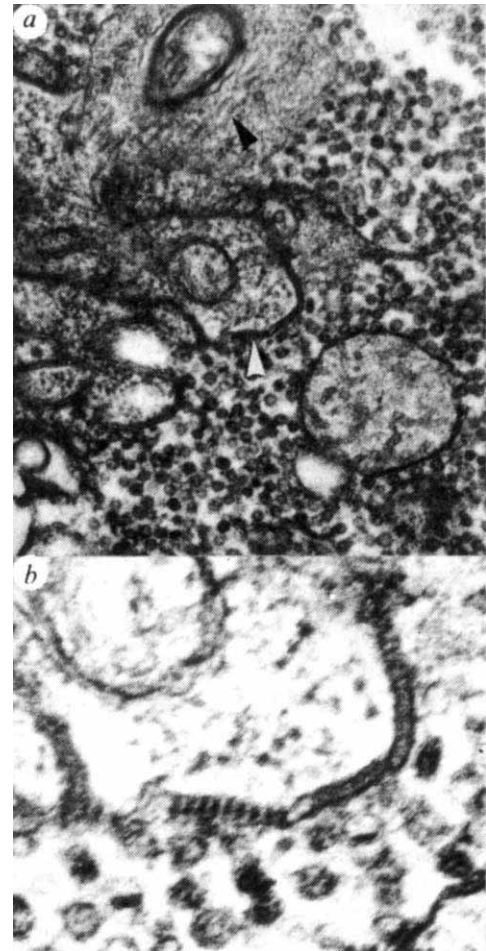
A small process of a growing neurone has been observed to form a septate junction<sup>15</sup> with a muscle cell, possibly a myoblast (Fig. 4). The neurone is characterised by the presence of numerous vesicles. The other structure has features characteristic of embryonic muscle cells; the



**Fig. 3** Growing region of axons (A) which is overlying an embryonic muscle cell. Two 'microspikes', filled with vesicles, are present (open arrows). The muscle cell nucleus (N) is at the bottom right. The sarcoplasm contains microtubules (filled arrows) and glycogen. A band of myofibrils (Mf) is present in the upper left. ( $\times 16,625$ .)

granular sarcoplasm contains mitochondria and microtubules. It is unlikely to be a glial cell as these have not been observed around the growing ends of the motor axons. In any case, septate junctions are not common features in the embryonic neuromuscular system. In the thirty preparations examined, extensive portions of growing axons were observed in five specimens, lesser portions in two other specimens. Only two septate junctions have been observed, however; thus these connections may be rare, transient<sup>1</sup> or both.

These results demonstrate that lobster motor axons can have two types of connections with muscle during embryogenesis. The growth cones of growing axons form septate junctions with immature muscle cells. This might well



**Fig. 4** Septate junction between a growing motoneurone and an embryonic muscle cell, probably a myoblast. The muscle cell has the characteristic granular sarcoplasm and microtubules (dark arrowhead). The neurone is filled with vesicles and has several small projections. *b*, An enlargement of the septate junction (white arrowhead) shown in the middle of *a* figure. (*a*,  $\times 31,540$ ; *b*,  $\times 92,960$ .)

be a period when the two different cell types can exchange chemical information. Later, when the muscle fibres have differentiated to a stage where sarcomere formation is well advanced, mature synaptic endings can be observed. Intracellular recording from these muscles has revealed that the synapses are indeed active. As early as the third month of development miniature excitatory postsynaptic potentials (e.p.s.ps) can be observed. Occasional spontaneous e.p.s.ps and inhibitory postsynaptic potentials have also been observed (in preparation). Thus it seems that both growth cones and active synapses may be present on these motor axons at the same time.



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## Is recombination confined to structural genes on the eukaryotic genome?

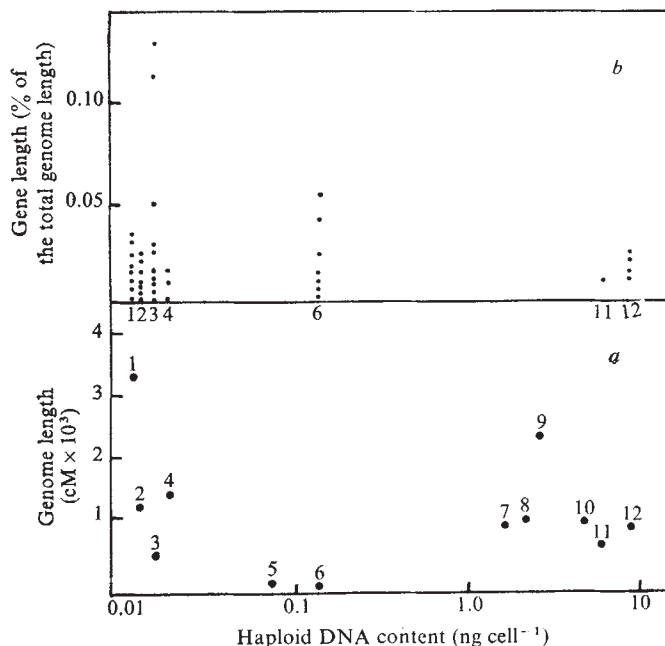
THE size of the eukaryotic genome increases with the evolutionary complexity of the organism, although this relation is somewhat obscured by large differences in the haploid DNA content of closely related species. Yet the total number of genes is believed to be fairly constant, suggesting that genes of higher eukaryotes correspond to much larger DNA segments than in microorganisms<sup>1</sup>. In that case, only a small fraction of each gene would code for the ultimate gene product, the remainder being 'spacer' DNA with still ill-defined functions. The sequence coding for the gene product will be hereafter designated as the structural gene. The complex organisation of chromatin in higher organisms raises the problem of its possible influence on the distribution of recombination events. I shall present here evidence suggesting that they may well be restricted to small regions of the genome, possibly corresponding to the structural genes.

The basic argument is that the length of total chromosome maps is fairly constant among eucaryotes (Table 1, Fig. 1) and that the same applies to intragenic fine-structure maps among the few organisms where they have been extensively investigated (Fig. 1b) such as several fungi, *D. melanogaster*, rice and maize. The conservation of the total recombinational length of eucaryotic genomes means that the average frequency of cross-over per unit length of DNA decreases by several orders of magnitude from lower to higher eukaryotes (Table 1, column 4). As opposed to intergenic crossover, intragenic recombination is mainly a non-reciprocal exchange. Yet, both seem to be related processes resulting from the formation of hybrid DNA molecules<sup>2,3</sup>. There are theoretical grounds to believe that they are either in the same, or in a 1/2 ratio with respect to actual distances<sup>4</sup>. If one further assumes that exchanges are randomly distributed on the genome and that intragenic maps correspond to the structural gene even in higher eukaryotes, the length of intragenic maps should considerably decrease from lower to higher eucaryotes.

The comparison of intragenic maps is complicated by two factors. First, recombination within a gene is not always proportional to actual distances, because of marker effects which are more or less prevalent depending on the organism investigated. Second, different regions of the genome have a different density of exchange per unit length of DNA, resulting in marked heterogeneities in the length of intragenic maps within one organism (Fig. 1b). On the whole, however, the length of

intragenic maps are roughly comparable in all eukaryotes investigated (Table 1, column 2 and Fig. 1b).

A key issue here is whether intragenic maps correspond to DNA stretches of comparable length in all eukaryotes. If intragenic maps were to include not only the structural genes but also the surrounding spacers, they would correspond to comparatively long DNA fragments in higher eukaryotes. This would compensate for the general decrease in the average frequency of recombination per unit length of DNA, and may result in the recombinational length of genes being fairly constant for all organisms. However, evidence suggests that point mutations in the spacers do not, as a rule, prevent gene expression (in other words, that intragenic maps are limited to the structural genes).



**Fig. 1** Recombination frequencies as a function of haploid DNA content. (a) Total genome length in centimorgans (cM). (b) Length of individual intragenic maps as a fraction (per cent) of the total genome length. Each point represents an individual gene map as given in Table 1, column 2. Haploid DNA content from refs 9, 10 and 11, except for *N. crassa*<sup>30</sup>, *A. nidulans*<sup>30</sup>, *C. radiatus*<sup>31</sup>, *C. elegans*<sup>23</sup>, *O. sativa*<sup>32</sup> and *P. sativum*<sup>40</sup>. The organisms represented are: *S. cerevisiae*<sup>1</sup>, *S. pombe*<sup>2</sup>, *N. crassa*<sup>3</sup>, *A. nidulans*<sup>4</sup>, *C. elegans*<sup>5</sup>, *D. melanogaster*<sup>6</sup>, *L. esculentum*<sup>7</sup>, *M. musculus*<sup>8</sup>, *H. sapiens*<sup>9</sup>, *P. sativum*<sup>10</sup>, *O. sativa*<sup>11</sup>, and *Z. mays*<sup>12</sup>.

First, it is established that spacers of rRNA evolve much more rapidly than the structural loci themselves<sup>5</sup> indicating, in that case at least, that there is no stringent requirement imposed on the spacer sequence. Second, work on the *rosy* locus of *D. melanogaster*<sup>8</sup> has shown full coincidence between an intragenic map constructed on 'null' mutants and another based on mutants with altered electrophoretic properties in the gene product, that is, mutants defining the structural locus. Similar evidence is available at two other loci of *D. melanogaster*<sup>7,8</sup>. Finally, the frequency of spontaneous forward mutation per locus per gamete is similar for yeasts, *D. melanogaster*, and mouse (Table 2) which suggests again that genes (defined as loci mutable to non-complementing alleles with a null phenotype) correspond to the same length of DNA in all eukaryotes.

On the other hand, the frequency of forward mutation per locus increases with the size of the genome when mutation is induced by X rays<sup>9</sup> or by ethyl methane sulphonate<sup>10</sup> (see ref. 11 for a critical evaluation of these data). X rays and perhaps EMS-induced intralocus mutants, however, are often deletions or equivalent multisite events in higher eukaryotes, whereas they are exclusively point mutations in fungi (see ref. 12). X rays do induce deletions in fungi, but these are multilocus events covering

**Table 1** Total linkage maps and intragenic maps among eukaryotes\*

| Organism                                            |                               | Length of intragenic maps (cM) | Total length | Average frequency of intergenic recombination (cM $\times$ kb <sup>-1</sup> ) |
|-----------------------------------------------------|-------------------------------|--------------------------------|--------------|-------------------------------------------------------------------------------|
| <i>Saccharomyces cerevisiae</i> (budding yeast)     | <i>ura2-A</i>                 | 0.12 <sup>21</sup>             | 3,600        | 0.26                                                                          |
|                                                     | <i>ura2-C</i>                 | 0.25 <sup>21</sup>             |              |                                                                               |
|                                                     | <i>ade3</i>                   | 0.80                           |              |                                                                               |
|                                                     | <i>arg4</i>                   | 1.16                           |              |                                                                               |
|                                                     | <i>ade8</i>                   | 1.25                           |              |                                                                               |
|                                                     | <i>his1</i>                   | 0.36                           |              |                                                                               |
|                                                     | <i>argB</i>                   | 0.37 <sup>22</sup>             |              |                                                                               |
|                                                     | <i>argC</i>                   | 0.60 <sup>22</sup>             |              |                                                                               |
|                                                     | <i>ade1</i>                   | 0.05                           |              |                                                                               |
|                                                     | <i>ade2</i>                   | 0.04                           |              |                                                                               |
| <i>Schizosaccharomyces pombe</i> (fission yeast)    | <i>ade4</i>                   | 0.13                           | 1,300        | 0.08                                                                          |
|                                                     | <i>ade6</i>                   | 0.05                           |              |                                                                               |
|                                                     | <i>ade7</i>                   | 0.10                           |              |                                                                               |
|                                                     | <i>ade8</i>                   | 0.01                           |              |                                                                               |
|                                                     | <i>ade9</i>                   | 0.35                           |              |                                                                               |
|                                                     | <i>his2</i>                   | 0.01                           |              |                                                                               |
|                                                     | <i>aro3</i>                   | 0.01                           |              |                                                                               |
|                                                     | <i>try1</i>                   | 0.28                           |              |                                                                               |
|                                                     | <i>try2</i>                   | 0.18                           |              |                                                                               |
|                                                     | <i>try3</i>                   | 0.05                           |              |                                                                               |
|                                                     | <i>lys1</i>                   | 0.04                           |              |                                                                               |
|                                                     | <i>cdc2</i>                   | 0.04                           |              |                                                                               |
|                                                     | <i>ade8</i>                   | 0.16                           |              |                                                                               |
|                                                     | <i>paba1</i>                  | 0.05                           |              |                                                                               |
|                                                     | <i>cho</i>                    | 0.30 <sup>27</sup>             |              |                                                                               |
| <i>Aspergillus nidulans</i>                         | <i>trp1</i>                   | 0.82                           | 600          | 0.05                                                                          |
|                                                     | <i>trp3</i>                   | 0.08                           |              |                                                                               |
|                                                     | <i>cys</i>                    | 0.30                           |              |                                                                               |
|                                                     | <i>pan2</i>                   | 0.70                           |              |                                                                               |
|                                                     | <i>met2</i>                   | 0.10                           |              |                                                                               |
|                                                     | <i>am</i>                     | 0.02                           |              |                                                                               |
|                                                     | <i>ade8</i>                   | 0.17                           |              |                                                                               |
|                                                     | <i>pyr3</i>                   | 0.03                           |              |                                                                               |
|                                                     | <i>arom</i>                   | 0.18                           |              |                                                                               |
|                                                     | <i>his3</i>                   | 0.06                           |              |                                                                               |
| <i>Neurospora crassa</i> (bread mould)              | <i>75</i>                     | 3.0                            | 360          | 0.004                                                                         |
|                                                     | <i>84w</i>                    | 1.2                            |              |                                                                               |
|                                                     | <i>46</i>                     | 0.6                            |              |                                                                               |
|                                                     | <i>w17</i>                    | 0.4                            |              |                                                                               |
| <i>Sordaria fimicola</i>                            | <i>G</i>                      | 0.04                           | 275          | 0.0017                                                                        |
|                                                     | <i>Coprinus radiatus ura1</i> | 0.03                           |              |                                                                               |
|                                                     | <i>and lagopus</i>            | 0.15                           |              |                                                                               |
| <i>Caenorhabditis elegans</i>                       | <i>ftr</i>                    | 0.15                           |              |                                                                               |
| <i>Tetranychus</i> sp. <i>Acarina</i> (spider mite) | <i>albino</i>                 | 0.20 <sup>24</sup>             |              |                                                                               |
| <i>Drosophila melanogaster</i> (fruit fly)          | <i>Notch</i>                  | 0.14 <sup>25</sup>             | 275          | 0.0017                                                                        |
|                                                     | <i>dp</i>                     | 0.14 <sup>25</sup>             |              |                                                                               |
|                                                     | <i>r</i>                      | 0.01 <sup>26</sup>             |              |                                                                               |
|                                                     | <i>bx</i>                     | 0.03 <sup>25</sup>             |              |                                                                               |
|                                                     | <i>w</i>                      | 0.026 <sup>25</sup>            |              |                                                                               |
|                                                     | <i>garnet</i>                 | 0.01 <sup>26</sup>             |              |                                                                               |
|                                                     | <i>rosy</i>                   | 0.005 <sup>16</sup>            |              |                                                                               |
|                                                     | <i>ma-1</i>                   | 0.001 <sup>7</sup>             |              |                                                                               |
| <i>Lycopersicon esculentum</i> (tomato)             |                               |                                | 1,100        | 0.0006                                                                        |
| <i>Mus musculus</i> (mouse)                         |                               |                                | 1,200        | 0.0006                                                                        |
| <i>Homo sapiens</i> (man)                           |                               |                                | 2,600        | 0.0009                                                                        |
| <i>Pisum sativum</i> (pea)                          |                               |                                | 1,200        | 0.0002                                                                        |
| <i>Oryza sativa</i> (rice)                          | <i>glt</i>                    | 0.10 <sup>27</sup>             | 750          | 0.0001                                                                        |
| <i>Zea mays</i> (maize)                             | <i>adh1</i>                   | 0.11 <sup>28</sup>             | 1,100        | 0.0001                                                                        |
|                                                     | <i>wx</i>                     | 0.25 <sup>14</sup>             |              |                                                                               |
|                                                     | <i>glt</i>                    | 0.12 <sup>15</sup>             |              |                                                                               |
|                                                     | <i>su1</i>                    | 0.20 <sup>29</sup>             |              |                                                                               |

\*In most cases, roughly linear maps can be constructed on the basis of two-factors crosses, thus supporting the assumption that intragenic frequencies of recombination are proportional to the actual distances on the gene and providing a direct estimate of the recombinational length of the gene. When necessary, recombination frequencies have been corrected for map expansion<sup>8</sup>. In some cases, the intragenic order of the alleles is quite ambiguous, and the recombinational length of the gene was assumed to be given by the heteroallelic combination giving the highest frequency of recombinant, though this need not define the largest distance in terms of base pair number. Total genome maps from ref. 20 except for *S. pombe*<sup>24</sup>, *N. crassa*<sup>18</sup>, *M. musculus*<sup>33</sup> and *H. sapiens*<sup>35</sup>. Except when not explicitly given, references for intragenic fungal maps are to be found in refs 2, 18 and 19, whereas data for *S. pombe* are either quoted in ref. 20 or come from unpublished work of Leupold and co-workers.

at least two genes and recoverable as recessive lethals in *N. crassa* dicaryons<sup>13</sup>. Thus, the differences in mutation rate could be due to deletions being retained as intralocus events as long as they remain within the boundaries of a single band in higher eukaryotes, whereas they are selected against in microorganisms where intergenic regions are quite short.

Hence, it can be assumed that even in higher organisms the actual length of intragenic maps is about 1000 base pairs, corresponding to the structural gene and perhaps a few regulatory sequences such as promoters. This gives a rough estimate of the frequency of intragenic exchanges per kb, which can be compared with the average frequency of intergenic exchange calculated over the whole genome (Table 1, column 4). In lower eukaryotes (Ascomycetes), the frequencies of intragenic and intergenic exchanges are of the same order of magnitude. This is what would be expected on the hybrid DNA model for recombination<sup>2-3-4</sup>. In higher eukaryotes, the density of intragenic exchanges is comparable with what is found in fungi, but much higher than the density of intergenic exchanges calculated over the whole genome. That is, intragenic maps are of similar length in all organisms, whereas the frequency of crossover per unit length of DNA considerably decreases from lower to higher eukaryotes (Table 1, column 4). This is very striking for higher plants (*O. sativa*, *Z. mays*) where intralocus maps are about 500 times larger than expected from the extrapolation of crossover frequencies. The same probably applies to *D. melanogaster* although the data are more dispersed and, therefore, more difficult to interpret: Five loci are 10–50 times longer than expected from the extrapolation of crossover data,

**Table 2** Frequency of spontaneous forward mutations per locus per gamete among eucaryotes (recalculated from papers quoted in ref. 12)

| Organism               | Screening system                                                                        | Frequency of mutation/locus /gamete |
|------------------------|-----------------------------------------------------------------------------------------|-------------------------------------|
| <i>S. pombe</i>        | Mutation to auxotrophy at five <i>ade</i> genes                                         | $1.6 \times 10^{-6}$                |
| <i>S. cerevisiae</i>   | Mutation to auxotrophy assuming 100 genes to lead to auxotrophic requirements           | $3.5 \times 10^{-6}$                |
| <i>D. melanogaster</i> | Recessive lethals on chromosome I, II and III assuming 4,500 genes on these chromosomes | $2.0 \times 10^{-6}$                |
| <i>M. musculus</i>     | Mutations at five coat colour loci                                                      | $1.8 \times 10^{-6}$                |

assuming an average gene to be 2 kb long (see also *albino* in spider mite). One locus (*ma-1*) is very short and would fit with a homogeneous distribution of intragenic versus intergenic exchanges. Two loci, (*garnet* and *rosy*, keeping in mind that *rosy* maps near the centromere in a region of unfrequent crossover) have intermediate values. On the whole, however, the data available for higher eukaryotes suggest a substantially greater frequency of recombination within, than between, the structural loci. If this is so, estimates of gene numbers in short regions of the genome such as the histo-incompatibility cluster of mammals should be revised, since the length of these regions (0.4 cM in mouse, see ref. 37) would be compatible with only a few genes.

A heterogeneous distribution of intragenic versus intergenic exchanges would have to be reconciled with the idea that both types of exchanges result from the formation of a hybrid DNA molecule<sup>2,3</sup>. One possibility is that, though hybrid DNA may occur with a roughly equal probability everywhere on the genome, strand isomerisation leading to crossover<sup>2,3</sup> is prevented by some features of the chromatine in higher eukaryotes. This would depress crossover without affecting non-reciprocal events. One would then expect, however, intragenic recombination to be predominantly associated with a parental distribution



of flanking markers, which is not observed in the few cases analysed so far in *Z. mays*<sup>14,15</sup> or *D. melanogaster*<sup>16,26</sup>.

I propose that this distribution of exchanges directly reflects the heterogeneous distribution of hybrid DNA on the chromosomes. That is, hybrid DNA formation may be mainly confined to the structural genes on the chromosomes of higher eukaryotes, in which case the frequency of exchanges per unit length of DNA should be much higher when calculated over short, intragenic distances than over the whole genome.

Cytological data show that the bulk of each chromosomal pair lies outside the synaptonemal complex and so cannot participate in recombination. It is not known whether this is randomly generated at each meiosis during the building up of the synaptonemal complex, or whether some mechanism restricts pairing always to the same stretches of the DNA, which may, for example, be regions with a dense distribution of structural genes. Alternatively, the necessity to keep the structural genes accessible to transcription may impose some basic constraint on chromatin organisation, resulting in them being preferentially accessible to hybrid DNA formation.

The fitness *ad equilibrium* of a polymorphic population is at a maximum in the absence of recombination<sup>17</sup>, which may account for the linkage maps being roughly constant despite the increase in genome length. As for intragenic recombination, it results in the formation of new alleles and, provided a reasonably high degree of gene polymorphism is assumed, may substantially affect the speed of gene evolution. Unlike mutation, however, its rate might be easily modulated in a site-specific way, assuming that the specific *rec* genes of *N. crassa*<sup>41</sup> are of general occurrence in eukaryotes. It may then have been selected as advantageous by organisms placed in a variable environment.

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## *n*-Butyrate causes histone modification in HeLa and Friend erythroleukaemia cells

LEDER and Leder<sup>1</sup> have reported that low concentrations of *n*-butyrate cause Friend erythroleukaemia cells to begin globin synthesis. Apparently *n*-butyrate can reverse that part of viral transformation which prevents the expression of differentiation in these cells. Prasad and Sinha<sup>2</sup> have summarised the effects of *n*-butyrate on neuroblastoma, HeLa, and other cell types. They and others<sup>3-10</sup> have seen reversible inhibition of proliferation, decrease of DNA content, morphological modifications, and increases in the production of specific enzymes, such as adenylate cyclase, alkaline phosphatase, and a sialyl-transferase. The present paper describes rapid, dramatic, and reversible increases in histone acetylation in the presence of *n*-butyrate.

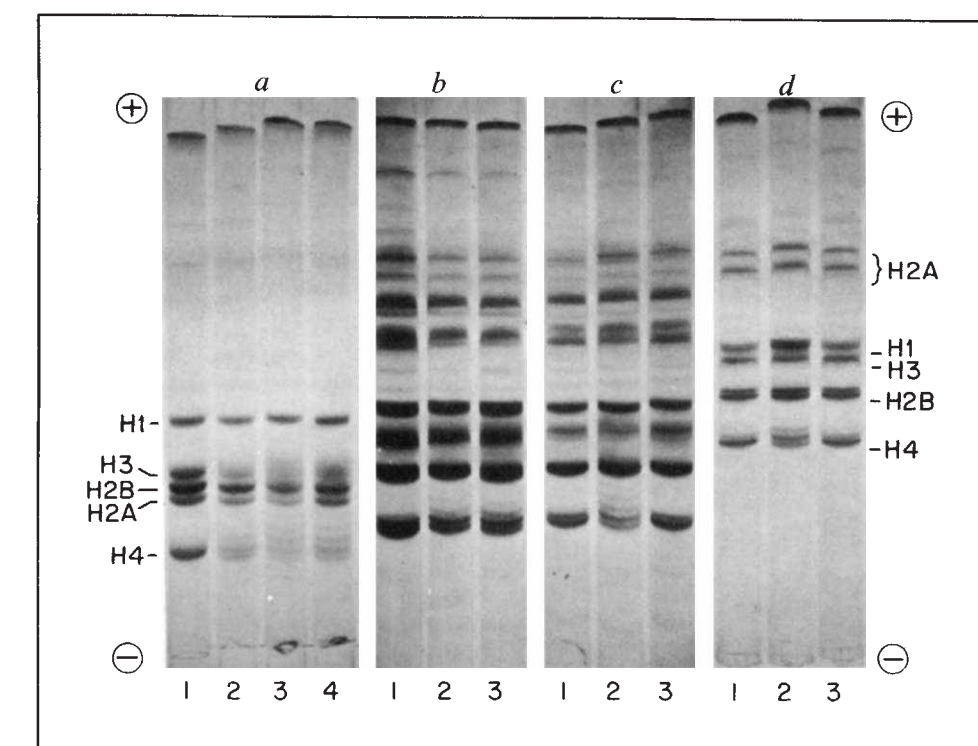
Nuclei from *n*-butyrate treated HeLa and Friend erythroleukaemia cells contain greatly increased amounts of modified forms of histone H4 (Figs 1, 2). Total histones from nuclei of cells after 24 h of treatment show several strong, slower moving H4 bands when examined in acid urea gels<sup>11</sup> and in Triton DF16-acid urea gels<sup>12</sup>. Their electrophoretic positions correspond to the mono-, di-, tri-, and tetra-acetylated H4 histones which occur during normal histone biosynthesis<sup>13</sup>. For this reason and because of the experiments described below, we believe the modified H4 histones to be acetylated. In control cells only a small amount of mono-acetyl-H4 is present, whereas this is the dominant component in treated cells. In HeLa cells the extent of modification, although marked at 1 mM, increases at higher *n*-butyrate concentrations (Fig. 1a). H4 acetylation and H3 modification in HeLa cells are already noticeable after only 1 h in 5 mM *n*-butyrate (Fig. 2), the earliest effect reported. Friend cells also show the acetylated-H4 pattern after 24 h in 1 mM *n*-butyrate; it becomes more pronounced at 3 mM (Fig. 1b).

In both HeLa and Friend erythroleukaemia cells the histone H4 pattern reverts to normal when cells treated with *n*-butyrate for 24 h are shifted back to control medium for a further 24 h (Fig. 1c, d).

In both cell lines there is also a clear alteration of the histone H3 band with an increase of slower moving material. This is a broadening of the H3 band rather than the appearance of new distinct bands. There is a striking increase in the relative amounts of H1 (Fig. 2). These changes are also reversible within 24 h after removal of *n*-butyrate.

To investigate the nature of the histone modification, total acid extracted histones from HeLa cells treated for 24 h with 5 mM *n*-butyrate were completely digested with trypsin and Pronase<sup>14</sup>. After separation from the proteolytic enzymes by Sephadex G25 column gel filtration, the free amino acids were analysed on a Durram analyser Model D500. A peak corresponding to  $\epsilon$ -N-acetyl-lysine was found just in front of glycine in the position of a standard prepared from acetylated bovine serum albumin<sup>15</sup>. This peak was absent in the sample prepared from control cells. No evidence for  $\epsilon$ -N-*n*-butyryl-lysine was found between alanine and valine in the position of an  $\epsilon$ -N-*n*-butyryl-lysine standard. The amount of  $\epsilon$ -N-acetyl-lysine was 1.7 mol per mol H4. It is not yet known whether these acetyl groups are all on H4 or whether they are more generally distributed. Quantitative analysis of the scans of gels shown in Fig. 2 indicated that the sum of the H4 components in *n*-butyrate treated cells was equal to the sum of the H4 components in control cells and that no H4 had been lost. The proportion of acetylated H4 reaches approximately 80% of total H4 at 24 h in 3 mM (Fig. 1a) or 5 mM *n*-butyrate (Fig. 2). In addition, we believe that the modification is

**Fig. 1** Effect of *n*-butyrate on histone modification in HeLa and Friend cells. HeLa cells were maintained at 37 °C in suspension culture at  $0.1\text{--}0.5 \times 10^6$  per ml in Joklik's modified minimum essential medium, containing 7% horse serum. Friend erythroleukaemia cells (clone 755-PC-4) (ref. 20) were grown at 37 °C in suspension in  $\alpha$  medium<sup>21</sup> lacking nucleosides and supplemented with 15% foetal calf serum. They were maintained at  $0.05\text{--}0.5 \times 10^6$  per ml to permit log-phase growth. Aliquots of a stock solution of 1 M Na *n*-butyrate in  $\text{Ca}^{2+}$ - and  $\text{Mg}^{2+}$ -free phosphate buffered saline (final pH 7.3) were added to cultures 24 h before histone extraction. Preparations of nuclei and histones were carried out at 0–4 °C. Nuclei were prepared from washed cells by Douncing and histones extracted<sup>22</sup> in 0.4 N  $\text{H}_2\text{SO}_4$ . *a*, Histones were precipitated with 10 volumes of acetone, dried and electrophoresed on acid-urea 15% acrylamide gels<sup>11</sup> at 20 °C for 5 h; the gels were prepared in 0.125% ammonium persulphate. *b*, *c* and *d*, histones prepared as before, were run on 15% gels containing 6M urea and 0.38% Triton DF-16, according to the method of Alfageme *et al.*<sup>12</sup>. Staining was with



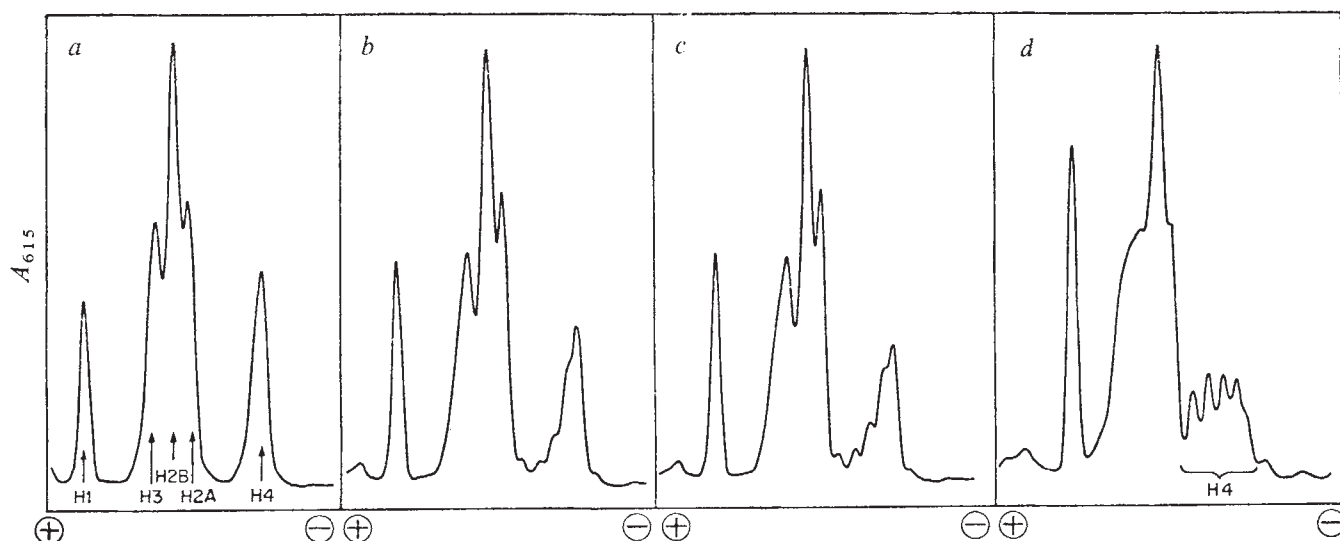
0.1% amido black in 40% methanol, 7% acetic acid. *a*, HeLa cells were either collected without *n*-butyrate treatment (gel 1) or after exposure to 1, 3, or 5 mM *n*-butyrate for 24 h (gels 2, 3, 4, respectively). *b*, Friend cells were collected without *n*-butyrate treatment (gel 1) or after exposure to 1 or 3 mM *n*-butyrate for 24 h (gels 2, 3, respectively). *c*, Untreated HeLa cells (gel 1), cells exposed to 5 mM *n*-butyrate for 24 h (gel 2), and 24-h *n*-butyrate-treated cells washed and resuspended in fresh medium without *n*-butyrate for 24 h (gel 3). *d*, Untreated Friend cells (gel 1), cells exposed to 1 mM *n*-butyrate for 24 h (gel 2) and 24-h *n*-butyrate-treated cells washed and resuspended in fresh medium without *n*-butyrate for 24 h (gel 3).

acetylation and not phosphorylation, since incubation of the modified histones with *Escherichia coli* alkaline phosphatase<sup>16</sup> leaves the modified H4 pattern intact.

We are particularly interested in the effect of *n*-butyrate on the proliferating malignant Friend erythroleukaemia cell and on the proliferating HeLa cell, which is also derived from a malignant cell. In the Friend cell differentiation is blocked. *n*-Butyrate induces the continuation of differentiation and the production of specific products, as well as the striking accumulation of acetylated histones. We do not know whether there is a causal

relationship between histone acetylation and these observations. Darzynkiewicz *et al.*<sup>17</sup> and Nudel *et al.*<sup>18</sup> have reported subtle changes in the structure of Friend cell chromatin using either dimethyl sulphoxide (DMSO) or *n*-butyrate as inducing agents.

It is not known whether *n*-butyrate affects tumorigenicity of the Friend cell or whether it affects DNA synthesis. If it behaves like the original inducing agent, DMSO, we would predict a decrease in tumorigenicity and, by analogy with our HeLa cell experiments (Hagopian, M.G.R., Swartz and V.M.I., in preparation), an



**Fig. 2** Time course of *n*-butyrate effect on HeLa histones. Nuclei were prepared and histones extracted as described in Fig. 1. Cells had been exposed to 5 mM *n*-butyrate for *b*, 1 h; *c*, 2 h and *d*, 24 h. Samples were electrophoresed on acid urea gels and stained as in Fig. 1. The gels were scanned at 615 nm with the full scale absorbances adjusted to normalise peak H2B. *a*, Control.



inhibition of DNA synthesis. Moore and Chalkley<sup>19</sup> using cultured hepatoma cells suggest an interdependence of the level of histone H4 acetylation and the rate of cell division. We do not observe acetylation changes in the histone patterns of DMSO-treated Friend cells (J.R.N., unpublished); perhaps the two agents act at different points of these complex processes. Further experimental work is needed to look for a causal relationship between the accumulation of polyacetylated histones in the presence of *n*-butyrate on the one hand, and the inhibiting of DNA synthesis and the switching of malignant cells to non-malignant differentiating cells on the other.

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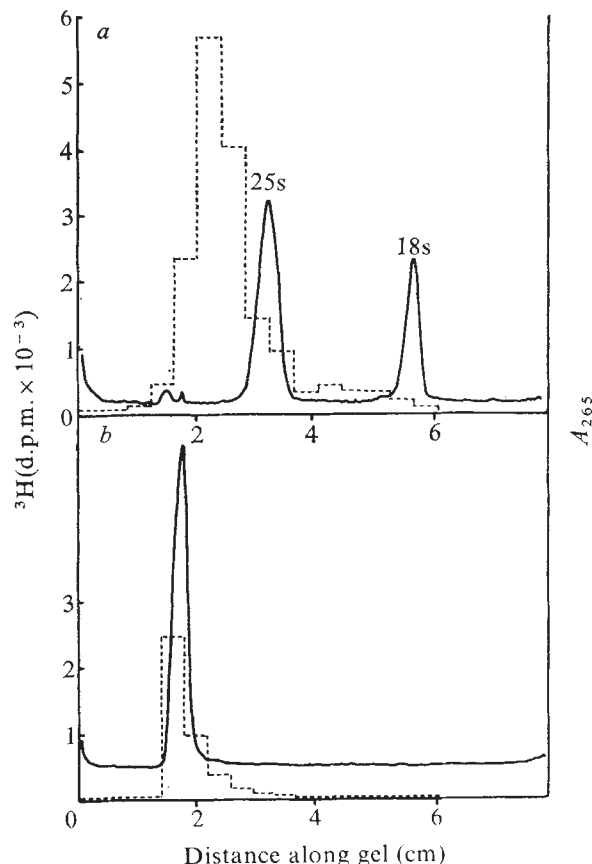
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## Yeast virus-like particles possess a capsid-associated single-stranded RNA polymerase

MANY species of fungi possess virus-like particles with double-stranded RNA (dsRNA) genomes<sup>1,2</sup>. In yeast the dsRNA genome has been shown to be the genetic determinant of the 'killer' character<sup>3–10</sup>. No cycle of lysis and reinfection has been demonstrated for 'viruses' of this group and replication is integrated with host growth<sup>1,2</sup>. Particles from several species of fungi have been shown to possess RNA polymerase activity and we have shown that yeast particles isolated from growing cells synthesise dsRNA (refs 11–14). We report here that particles isolated from non-growing cells synthesise both dsRNA and a single-stranded (ssRNA) species with properties suggesting that

it is a complete transcript of the dsRNA genome. Our results with particles from growing cells suggested that the replicative cycle of yeast 'virus' resembles that of reovirus<sup>14</sup> and does not occur through the 'doubling cycle' which was put forward by Buck<sup>13</sup> to account for his results with *Penicillium stoloniferum* S. virus. The discovery of a ssRNA polymerase activity in yeast particles is consistent with the reovirus type of replicative cycle.

Particles were prepared from stationary phase cells of the strain 3/A1 (ref. 3) which possesses only P1 dsRNA and were incubated in the RNA polymerase reaction mixture as described in the legend to Fig. 1. The RNA reaction product was isolated and separated into ssRNA and dsRNA fractions by chromatography on cellulose<sup>14,15</sup>. These fractions were analysed by polyacrylamide gel electrophoresis (Fig. 1). Incorporation was found in both fractions but only 4%



**Fig. 1** Analysis of the ssRNA polymerase product on 2.6% polyacrylamide gels<sup>27</sup>. Yeast VLPs were prepared from strain 3/A1 after 10 d of culture by disruption of the cells with glass beads followed by polyethylene glycol precipitation of the VLPs (ref. 21) and isolation by differential and sucrose gradient centrifugation<sup>1</sup>. 2.9  $A_{260}$  units of VLPs were incubated at 30 °C for 125 min in 0.7 ml of 0.05 M Tris-HCl buffer (pH 7.9) containing 0.15 M KCl; 0.1 mM EDTA; 3.0 mM  $MgCl_2$ ; 0.20 mM GTP; 0.20 mM ATP; 0.20 mM CTP; 0.20 mM  $^3H$ -UTP (330 mCi mmol<sup>-1</sup>) and bentonite (2 mg ml<sup>-1</sup>)<sup>13</sup>. RNA was prepared by addition of 2 volumes of 0.05 M Tris-HCl containing 0.1 M NaCl and 2% tri-isopropylphenyl sulphate followed by two extractions with phenol and alcohol precipitation with 100  $\mu$ g of yeast tRNA as carrier. CF-11 cellulose chromatography<sup>15,16</sup> was used to separate the ssRNA and dsRNA followed by reprecipitation and analysis on gels. Figure 1a shows the  $A_{265}$  (solid line) and radioactivity profiles (dotted line) of a gel loaded with 1/7th of the total ssRNA product from the reaction mixture described above plus 30  $\mu$ g of total yeast nucleic acid. Figure 1b shows similar profiles for the total dsRNA loaded on to the gel after digestion with ribonuclease A (1.0  $\mu$ g ml<sup>-1</sup>) in 1×SSC (0.15 M NaCl, 0.015 M Na citrate, pH 7.0) for 10 min at 17 °C. The radioactivity profile was obtained by slicing the gel into 4-mm slices, digesting the slices with 0.5 ml  $H_2O_2$  overnight at 60 °C and scintillation counting after addition of 5 ml of scintillation fluid (Nuclear Enterprises NE 250) to each vial.

of the counts were found in dsRNA. The major ssRNA product migrates as a single species with an estimated molecular weight of  $1.6 \times 10^6$  relative to yeast ribosomal RNA standards<sup>16</sup>. This value is in good agreement with the estimates of the molecular weight of denatured P1 dsRNA obtained by polyacrylamide gel electrophoresis in formamide, which were  $1.60$  and  $1.63 \times 10^6$ , but is not consistent with our reported estimate of  $2.5 \times 10^6$  for P1 dsRNA, estimated from aqueous gels<sup>16</sup>. The molecular weights of the *Aspergillus foetidus* S. virus dsRNA, however, which we used as standards have been re-estimated as  $4.0 \times 10^6$  and  $2.5 \times 10^6$  (personal communication from K. W. Buck and G. Ratti). This has enabled us to revise our molecular weight estimate for P1 dsRNA to  $3.3 \times 10^6$ . This figure agrees well with the formamide gel data.

The inclusion of bentonite in the reaction mixture is necessary to eliminate contaminating ribonuclease activity<sup>17</sup>; without bentonite the ssRNA product was found to be of low molecular weight. Because a low molecular weight product was found when VLPs from growing cells were incubated without bentonite<sup>14</sup> it is probable that they too possess ssRNA polymerase activity.

The gel profile of the dsRNA labelled in the reaction differs from that made by VLPs from growing cells in that it was of normal genome size and its mobility was unaltered by ribonuclease digestion (undigested sample not shown). This can be explained in three ways: it may be the result of the completion of short unreplicated ssRNA 'tails' on dsRNA molecules, as suggested for the dsRNA polymerase activity found associated with bacteriophage  $\Phi 6$  virions<sup>18</sup>; it could be produced by a doubling reaction in which a small proportion of the ssRNA transcripts become templates for the dsRNA polymerase as is thought to occur in *P. stoloniferum* S. particles<sup>13</sup>; or it may be that the mechanism of the transcription reaction is not completely conservative as in reovirus<sup>19</sup> but semi-conservative with the ssRNA product being produced by strand displacement. This latter mechanism is thought to operate *in vivo* during the replication of bacteriophage  $\Phi 6$  (ref. 20). We are attempting to distinguish these alternative mechanisms experimentally.

The first report of a fungal virus polymerase producing ssRNA was from Ratti and Buck<sup>21</sup>, who found that 70% of the *in vitro* product formed by particles of *Aspergillus foetidus* S. virus was sensitive to ribonuclease while the remainder was resistant and was assumed to be dsRNA. If the mechanism of the transcriptase reaction is strand displacement, the ratios of ds to ssRNA synthesised both in yeast and *Aspergillus foetidus* particles suggests that only in a few active particles which can initiate several rounds of synthesis does the reaction occur.

That the  $1.6 \times 10^6$  molecular weight ssRNA product is a faithful transcript of the P1 dsRNA genome was substantiated by the hybridisation data shown in Fig. 2. The annealing conditions used, which are described in the legend to Fig. 2, are based on those of Kielland-Brandt and Nilsson-Tillgren<sup>22</sup>. An excess of unlabelled P1 dsRNA was denatured and reannealed in the presence of ssRNA polymerase product. The renatured RNA was digested with ribonuclease and analysed on polyacrylamide gels. Fig. 2a shows that the peak of activity and the peak of accurately renatured dsRNA coincide in the gel, that is, the ssRNA product is very near to half genome size as is the case with reovirus transcripts hybridised to genome dsRNA (ref. 23). The trailing shown by the peak of activity down the gel was also seen when the gel was stained although it is not very clear from the  $A_{265}$  profile. It is due to the heterogeneous population of dsRNA molecules which are generated by the digestion of network structures formed during the annealing<sup>23</sup>. Figure 2b shows a control sample which was not subjected to denaturation but was simply incubated at the annealing temperature. No activity is found on the gel which confirms the ribonuclease sensitivity

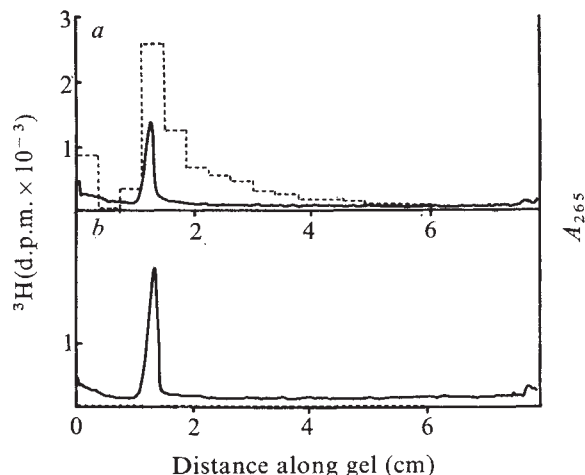


Fig. 2 Hybridisation of the ssRNA polymerase product. Polymerase product was taken up in 2.0 ml of  $1 \times$  SSC containing 45% (v/v) formamide and  $20 \mu\text{g}$  of unlabelled P1 dsRNA. The sample was divided equally, sample (a) was heated to  $85^\circ\text{C}$  for 3 min and then incubated at  $60^\circ\text{C}$  for 30 min, sample (b) was incubated at  $60^\circ\text{C}$  for 33 min. Both samples were precipitated with 2 vol. of ethanol after the addition of  $100 \mu\text{g}$  of tRNA as carrier and loaded on to 2.6% polyacrylamide gels<sup>27</sup> after a 10 min digestion with ribonuclease as described for sample (b) in Fig. 1. Electrophoresis was for 6.5 h at 3.8 mA per tube.  $A_{265}$ , solid line; radioactivity, dotted line.

expected of ssRNA and shows that it does not form ribonuclease resistant dsRNA by a process of self-annealing. The ssRNA product thus seems to be a transcript of a single strand of the dsRNA.

The amount of input ssRNA entering hybrid was calculated from the results of a separate experiment at 69%. This experiment included a sample which was neither denatured nor ribonuclease-digested. The degree of renaturation in this experiment, calculated by a comparison of the area of the dsRNA peaks in the  $A_{265}$  profiles, was found to be 65%.

The data together with our previous results show that both the polymerase activities required to undergo the asynchronous cycle of replication utilised by reovirus<sup>19</sup> are found in the yeast VLP. Bruenn and Keitz<sup>24</sup> have shown recently that yeast dsRNAs lack the 7-methyl guanosine 'cap' structures which are found both at the 5' end of the + strands of reovirus dsRNAs<sup>25</sup> and in yeast mRNAs (ref. 26). They have suggested that this indicates that replication does not proceed by the reovirus cycle. We are investigating the *in vitro* methylation of the ssRNA polymerase product together with other aspects of its structure and synthesis.

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## Enzymology in supercooled water

RECENT years have witnessed an increasing interest in applications of low temperature techniques to studies of enzyme-catalysed reactions, the aims being the temporal resolution of the contributing reactions and the stabilisation of intermediate species. To overcome the low temperature limit set by the freezing point of the aqueous medium mixtures of water and various polar organic solvents have been used<sup>1-4</sup>. Although these aqueous mixtures affect the solvation interactions of the enzyme and its conformational stability<sup>5</sup>, they can be shown to influence neither substrate specificity, nor the reaction pathway. We have investigated the feasibility of supercooled water to achieve conditions closer to the physiological ones. To circumvent freezing by heterogeneous nucleation, the aqueous phase containing enzyme, substrate and buffers was emulsified in an oil which was supersaturated with a water insoluble surfactant<sup>6</sup>. We here present the results on bacterial cytochrome  $P_{450}$ , a mono-oxygenase which has already been studied in mixed solvents<sup>1,2</sup>.

Sorbitan tristearate was added to corn oil or safflower oil at 2.5% (w/v) and dissolved by gentle heating. An aliquot (1.5 ml) of an aqueous solution containing the enzyme in phosphate buffer, KCl and camphor (substrate) was emulsified in 3.5 ml of the oil phase at 10 °C. This was achieved by manual shaking, followed by microemulsification in a blender, until a droplet size of 1-5 µm diameter had been obtained. Optical spectra were recorded at temperatures down to -30 °C using an Aminco-Chance DW2 spectrophotometer. The various redox states of the substrate bound enzyme could be observed at low temperatures. The equilibrium constant governing the spin state equilibrium of the ferric cytochrome  $P_{405}$  was measured at several temperatures. The Van't Hoff  $\Delta H$  at pH 7 agreed well with the corresponding value at ambient

temperatures (10.3 kJ mol<sup>-1</sup>) (ref. 7) but differed significantly from the low temperature value (30.1 kJ mol<sup>-1</sup>) determined in a 50% aqueous ethylene glycol solution<sup>8</sup>.

To investigate the possible stabilisation of labile species at subzero temperatures the unstable compound  $P_{450}$  ( $\text{Fe}^{2+} \cdot \text{O}_2$ ) was formed at 0 °C, quickly emulsified and cooled to -20 °C in the spectrophotometer. The absorption spectrum in Fig. 1 clearly shows the maxima at 418 and 552 nm, characteristic of the oxy-ferrocycytochrome. Repetitive spectra taken over a period of 1 h showed no evidence of autoxidation. After heating the emulsion to 25 °C for 20 min and recording another spectrum at -20 °C, the compound had been totally transformed to  $\text{Fe}^{3+}$ . All spectra recorded in supercooled water were identical to those observed in normal conditions, except for a low temperature-induced sharpening of the peaks.

The preliminary experiments indicate that the extension of the accessible temperature range to the homogeneous nucleation temperature (approximately -40 °C) permits the stabilisation of enzyme-substrate complexes, and of labile intermediates and makes possible the temporal resolution of the enzyme catalysed reaction. A more detailed account of this work is in preparation.

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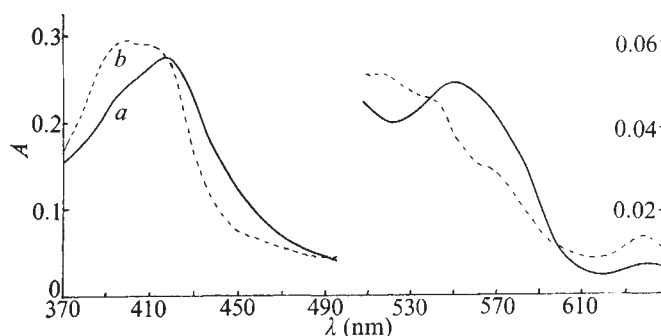


Fig. 1 Spectra of (a) oxy-ferrocycytochrome  $P_{450}$  ( $\text{Fe}^{2+} \cdot \text{O}_2$ ) stabilised at -20 °C in emulsified supercooled water, and (b) the same sample after warming and maintaining at 20 °C for 40 min and subsequently recooling to -20 °C. The long wavelength part of the spectrum (right-hand ordinate) was recorded with a higher sensitivity.

## Corrigenda

In 'Lead isotope measurements from the oldest recognised Lewisian gneisses of north-west Scotland' by H. J. Chapman and S. Moorbath (*Nature* **268**, 41; 1977), line 4 in paragraph four should read . . . The isochron yields an age of  $2680 \pm 60$  Myr ( $2\sigma$ ), which . . .

In 'Eighty thousand  $\beta$ -adrenoreceptors in a single cell' by D. Atlas, F. Hanski, A. Levitski (*Nature*, **268**, 145; 1977) the ordinate label of Fig. 1c should read . . . ( $\text{M}^{-1} \times 10^{-14}$ ).

In 'Histone content of germinating pea embryo chromatin decreases as DNA replicates' by F. Grellett, M. Delsený and Y. Guitton (*Nature* **267**, 724; 1977) the labelling of the peaks in Fig. 1 should read (from left to right): H1 (30,500; 26,500; 24,800), H2B (16,800), H2A (15,100), H3 (13,500), H4 (10,500).

# reviews

## A zoophile's testament

Eric Ashby

*The Moral Status of Animals.* By Stephen R. L. Clarke. Pp. 221. (Oxford University: Oxford and London, 1977.) £5.95.

IN fairness to the author of this book, it must be said that he is a professional philosopher and this reviewer is not. And in fairness to this reviewer it must be said that the book is not addressed to philosophers but to the public at large. Moreover, it is not written to inform: it is written to persuade, with an uncompromising vehemence unusual among philosophers. So Dr Clark has to put up with the fact that I'm not competent to comment on the subtleties of his logic, just as I have to put up with Dr Clark's assertion (p183) "that no-one has any standing" in discussing the theme of his book "who has not taken the simple, minimal step of abandoning flesh-foods."

For that is the demand—and 'demand' is not too strong a word—which Dr Clark makes upon the reader. "I am a committed crank and zoophile, and my hope is to convert my audience." "Those who still eat flesh when they could do otherwise have no claim to be serious moralists." Dr Clark is fully aware of the consequences which would follow if his mission were to succeed. There would be a phasing out of all animal husbandry, from sheep and poultry, to fisheries and (presumably) shellfish beds. The sight of sheep placidly grazing is offensive to Dr Clark; they are the innocent and unaware "inmates of our Belsens" (p80). If this seems immoderate and emotive language from the pen of a philosopher, Dr Clark defends himself with the statement: "I am very much more interested in achieving a practical issue than in the details of abstract philosophy, and am willing to argue on almost any basis to achieve that end." But even this defence does not excuse such nonsense as the assertion (p188) that "flesh-eaters pretend that their victims delight to serve them."

Of course, abstinence from all flesh foods is only one of the demands which Dr Clark makes upon us. There must also be abstinence from all vivisection and from the use of animals to test drugs, on the ground that we must not do to animals anything which we would not legitimately do to human beings.

The book is written in a learned, not to say pedantic style, with copious references to authorities from Porphyry in the third century AD to Roszak in the twentieth century. Dr Clark professes himself to be a Christian but he confesses to finding little support for his thesis in the Bible and he is embarrassed by Christ's participation in the catching of fish. He admits that there is some sort of hierarchy among living creatures—to murder a sheep is "perhaps in less degree" serious than to murder a shepherd—and he is evasive about insects, although he admits with reluctance (p82) that insects killed on impact with a car windscreen weigh "less heavily than a deliberate killing."

There is an appendix with advice on vegetarian cooking.

This is a deeply disappointing book, for the issues which Dr Clark discusses are important. A critical examination of them would be timely because—contrary to numerous reckless assertions in Dr Clark's book—the common man is aware as never before of his kinship with the rest of nature. No-one who has seen nature films on TV believes (as Dr Clark implies on p37) that animals are stupid; no-one doubts that they suffer pain, can be apprehensive of danger, and that many kinds of animals are capable of affection, not only within their species but even toward man.

The widespread empathy between human beings and the rest of the natural world creates an interest in the rights of animals. A careful and sober enquiry into the consequences of this

empathy would be welcome. Any such enquiry would have to take into account three considerations, none of which Dr Clark has faced squarely.

First, if as Dr Clark admits, the intensity of concern diminishes as one passes from mammals to vertebrate and from vertebrates to insects, where does it stop? And if not at insects, why not include plants? (Christopher Stone has already written an essay, far more persuasive than Dr Clark's, on the question whether trees should have standing in the law-courts of America.)

Secondly, Dr Clark admits that it's not our business to save the rabbit from the fox or the fly from the spider, but he does not explain why man, as an integral unit in nature, should abdicate from the place he has occupied in the food chain for millennia. Hamlet's "We fat all creatures else to fat us and we fat ourselves for maggots . . ." is a curt but accurate summary of man's place in the biosphere.

Finally, much of Dr Clark's case rests on the premise that man must not treat animals with less concern than he treats his fellow men. In fact man treats animals with more concern: we may kill domesticated animals in order to eat them; we don't torture them because they do not share our political or religious ideologies. The intraspecific behaviour of animals to one another is, by and large, more compassionate than our own intraspecific behaviour. □

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## Cell surface in embryogenesis

*Cell Surface Reviews.* Volume 1: The Cell Surface in Animal Embryogenesis and Development. Edited by George Poste and Garth L. Nicholson. Pp. 766. (North-Holland: Amsterdam, New York and Oxford, 1977.) \$89.95; Dfl.220.

THIS is the first in a new series of volumes designed to collate literature on cell membrane research. Three more volumes (Virus Infection and the Cell Surface, Dynamic Aspects of Cell Surface Organisation, Membrane As-

sembly and Turnover) are promised within the year. After this, volumes will be added to the series annually. This book deals with the more general aspects of development.

The chapter by Trinkaus is a scholarly survey of cell movement. Widely held misconceptions are exposed and corrected. Arnold's review on cytokinesis is concise and lucid.

Cell communication is discussed in four of the reviews. Sheridan provides circumstantial evidence for gap junctions as a structural basis for low



resistance coupling of cells. Uncoupling of cells in development may prove to be an exciting clue to the mechanism of control. Aspects of differentiation relating to cell or tissue position are examined in a splendidly iconoclastic spirit (McMahon and West) in both regulating and non-regulating systems. Most of the important research dealing with embryonic induction has been compiled by Saxén *et al.* Cell surface specificity in cell systems *in vitro* seems to be present (Maslow), but its relevance to actual developmental events can always be questioned.

Five of the reviews deal with the development of the mammalian conceptus and placenta. They might have been more usefully placed in one of the later volumes. Gwatkin restricts his consideration of the organisation of the cell surface of gametes to the mouse. This seems a pity since much of the interesting work in this field lies in invertebrate studies. Sherman and Wudl make provocative comments

about the putative role of the blastocyst in implantation. The transport of molecules across placental membranes is lengthily listed (Miller, Koszalka and Brent) and some studies of cell surface antigens in mammalian developments are reviewed by Edidin.

The development in only three organ systems is covered, and in each a somewhat pedestrian note is struck. Hot or controversial issues have not been stirred up in these reviews about the heart (Manasek), limb bud (Ede), or immune system (Goldschneider and Barton).

All in all, the editors and reviewers have done a fair job. The publishers, however, can be severely criticised. The book costs ninety dollars, has many typing errors and is inadequately indexed. I have recommended this book to our library, but they cannot afford it.

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## Solar planets

*The Solar Planets.* By V. A. Firsoff. Pp. 184. (David and Charles: Newton Abbot, London and Vancouver; Crane, Russak: New York, 1977.) £5.50.

CRUDELY one can divide science books into four categories, elementary and advanced, good and bad. Firsoff's book is elementary and bad.

There is nothing wrong of course with a book being elementary. This one is written for the amateur astronomer and purports to be a study of the Solar System written in the light of the wealth of new knowledge gleaned from the unmanned space probes which have recently visited Mercury, Venus, Moon, Mars, Jupiter and Saturn. True, the book contains plenty of details about these missions, seventeen black and white plates and no formulae. And obviously the book is not all wrong, in fact a good 95% is right, but the unsuspecting reader has no way of picking out right from wrong, fact from the authors' flight of fantasy, the solid ground from the castles in Spain.

We are told: "it seems that the Moon began its existence as an independent planet and was later captured by the Earth", "a limestone meteorite fell in 1926 at Bleckenstad and is said to have contained fossils"; "Pluto may possibly belong to an older solar system or else be an immigrant from interstellar space"; "degeneracy is a condition derived from equations based on various simplifications which are always suspect". "I am not too sure that even in the Solar System ours is the only technical civilisation", "geography is fast becoming only a division

of astronomy"; "fissile radioactive materials could become concentrated by geological processes into a natural atomic pile, which could occasionally blow up", and a host of other *non sequiturs*. Many facts pop out at us like rabbits from a hat accompanied by a similar aura of surprise and lack of explanation. The book also uses jargon freely. The reader, having no glossary, has to turn to other works for definitions of such terms as Hohmann transfers, coriolis force, grey-sphere radiative equilibrium, body tides, bond albedo and so on. Also in evidence is Firsoff's dislike of professional scientists. For example, "the idea of an inert Moon is still widely held among scientific officialdom, often in the teeth of overwhelming evidence against it". In discussing a dark pattern seen on one of the rocks on Venus pictured by Venera 9 Firsoff puts forward the idea that this could be a lichen growth—a genus from a high temperature biology based on sulphuric acid as the solvent: "of course this is speculation" Firsoff writes, "and speculation is a dirty word in professional circles—but many of the hypotheses that have been given an official accolade are even less well founded".

This book gives an entirely wrong impression of present knowledge of the Solar System and of the state of mind of the scientific enquirer who is presently investigating the planets. Not only do I advise people not to buy this book, I will go further, don't even bother to open it.

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## Pursuit rotor performance

*Reminiscence, Motivation and Personality.* A Case study in Experimental Psychology. By H. J. Eysenck and C. D. Frith. Pp. xxi+430. (Plenum: New York and London; 1977.) \$33.

THIS self-proclaimed "case study in experimental psychology" is concerned exclusively with the phenomenon of reminiscence as observed in studies of pursuit rotor performance. The pursuit rotor is a piece of apparatus which requires a human subject to keep a stylus in contact with a small target on a rotating turntable. The reminiscence effect refers to a gain in performance—and possibly in learning—when a rest period is introduced after a period of practice. The authors' introduction asserts that "whether the accumulated work done by generations of students on the problems discussed in this book has been worth the trouble must be left to the reader to judge." The authors are presumably optimistic about the reader's verdict; but not, I think, with good reason.

The major virtue of the pursuit rotor is said to be the replicability of the data which it generates—"perhaps the most reliable set of observations in experimental psychology." The pursuit rotor, we are told, is "the psychologists' favorite piece of apparatus." These claims seem absurdly parochial, but even if taken seriously they are offset by the authors' remark—in criticising a study which disagrees with their views—that "the literature on reminiscence is especially rich in 'failures to replicate.'"

The book does, however, provide a detailed history of research on this narrow problem. The same studies which the authors had earlier interpreted in terms of "inhibition" theory are now interpreted in terms of "consolidation" theory. The fit of the new theory to data, like that of the old, is not so striking as to compel belief. The theoretical concepts used have not arisen from the study of reminiscence in the pursuit rotor task, but have been imported from general psychology. There is no shortage of available 'explanatory' concepts.

This work will be of interest, and possibly of value, to those who are concerned with the pursuit rotor and related matters. There are, however, many experimental psychologists who would have preferred a more fertile subject for such extensive case study.

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## Regge theory

*An Introduction to Regge Theory and High Energy Physics.* By P. D. B. Collins. Pp. xiii+445. (Cambridge University: Cambridge, London and New York, 1977.) £29.

OVER the past fifteen years, the theory and phenomenology of high energy collisions of strongly interacting particles have developed into a powerful and coherent picture. The basic idea is a subtle generalisation of Yukawa's idea of forces being due to exchanges of particles. This idea is, of course, built on the classic example of electrodynamics, where exchange of photons provides the electromagnetic forces between electrons. When this idea is applied to the exchange of known strongly interacting particles of high spin it leads to absurdities in specific kinematic regions. The classic mathematics of Regge and its applications by Chew, Frautschi, Mandelstam and others was that an object called a Regge pole could be usefully defined, which subtly changed from being one particle into another for different kinematics. Thus, exchange of a Regge pole is equivalent to exchange of many Yukawa particles in a simple way. The Regge pole also clearly serves to classify the several (many?) particles whose exchange it controls. These particles all have different spins. Thus, it provides a classification of a totally different nature to the canonical isospin, strangeness ( $SU(2)$  and  $SU(3)$ ), beloved of group theorists, which classifies objects of the same spin.

This theory has been developed and tested in a wide variety of collision processes and has encountered no conspicuous disasters in confronting the data. The status of experiment and theory in this field are superbly reviewed by Dr Collins in this elegant book. The level is beautifully judged to lead a new research student into the depths of this field and start him on his research career. On the other hand, as a worker in this field, it gave me great pleasure to see the material laid out so coherently. The book contains a judicious amount of data without which one can so easily become lost in the theoretical intricacies.

After a quick introductory review of the basic assumptions of scattering theory, Dr Collins turns to the fundamental derivations of the complex angular momentum plane and models which show the existence of Regge poles. The basic Regge trajectories are then discussed and the connection with internal symmetry groups brought out in detail. These trajectories are found from fitting the data. The details of these fits are admirably described.

Because of analyticity, if we know the high energy behaviour of scattering amplitudes, strong constraints are imposed by Cauchy's theorem on the low energy amplitudes. These constraints are typically given by the so-called finite energy sum rules and duality. These are again succinctly described by Dr Collins.

In a relativistic theory it is known that as well as the Regge poles to be exchanged there are so-called 'Regge cuts' which come from exchange of two Regge poles. These correspond to re-scattering cross-sections in the manner of Glauber theory. This is an area of great theoretical interest in view of the rising total cross-sections found at the CERN intersecting storage rings using the highest available energies. This chapter is followed by a discussion of the ingenious way in which Regge poles contribute to collisions with three or more particles in the final state. In particular, the application to inclusive processes where, say, two protons collide to turn into a pion plus an unknown number of

other final state particles, is described in detail. This is a true success of the theory in a fundamentally relativistic situation, with many particles being produced out of pure energy.

Finally, the relatively unsuccessful attempts to calculate the Regge trajectories from more fundamental theories are described. This joins naturally to the last chapter which connects with conventional field theories and their Regge trajectories. A brief discussion of deep inelastic electron-proton scattering is given, with its hint that there may be point constituents in protons, unlike the fuzzy picture suggested by Regge theory.

In conclusion, this is a superb introduction to strong interaction dynamics with an admirable blend of theory, speculation and experimental data which I cannot recommend too warmly.

I. G. Halliday

*I. G. Halliday is Reader in Theoretical Physics at the Blackett Laboratory, Imperial College, University of London, UK.*

## Mercurial chemistry

*The Chemistry of Mercury.* Edited by C. A. McAuliffe. Pp. 288 (Macmillan: London, 1977.) £25.

WHILE reading this slim volume one is conscious of the fact that each page costs just under 10p and it is thus rather annoying to find that 29 pages are completely blank, contain only titles or are less than half full.

Unhappily the book falls far short of its title; certainly it is not a treatise on mercury as claimed in the Introduction. There is no attempt made to compare mercury with its congeners or with the alkaline earth elements, and the aqueous chemistry of  $Hg^{2+}$  is dismissed in the eight lines of "chapter" 17. I had hoped that one could have picked up this book and had the whole of mercury chemistry at one's finger tips but, since the style of the main chapters is somewhat similar to that of the *Annual Reports of the Chemical Society*, access to a good library is essential to obtain sufficient background knowledge. Although "to avoid duplication, work presented in recent reviews is not duplicated here", there is considerable overlap in Chapters 11 through 17 with Professor Aylett's article (about 1969) in *Pergamon's Comprehensive Inorganic Chemistry*—indeed, in several places the two descriptions of  $Hg_2^{2+}$  and  $Hg^{2+}$  chemistry, necessarily, read almost word for word; naturally this book contains a little

more up-to-date material (May, 1975). I looked for, but did not notice, any mention of Aylett's review among the copious references.

The first part of the book gives a readable account by Farrar and Williams of the history of mercury, but takes up 45 precious pages; numerous quotations from translations of Pliny and alchemical texts are presented. Part two by Levason and McAuliffe discusses the coordination chemistry of  $Hg_2^{2+}$  and  $Hg^{2+}$ . The organic chemistry of Hg (II) is dealt with in considerable detail by Bloodworth in Part three, and the biochemistry and toxicology of mercury (Falchuk, Goldwater and Vallee) are dismissed in a disappointingly brief section considering their current intense interest.

There would seem to be no justification for a short book of this title, and certainly the price will keep it out of the reach of all but the most dedicated of mercury chemists and the well-endowed libraries. On reading the title I had high hopes as to the contents but these hopes were largely shattered as it became clear that the book was meant as a review and not a readable and critical account of this most unique element. Approximately two dozen actual printing errors were noticed, whereas broken type occurred with too high a regularity for a book in this price range.

A. G. Massey

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## Structure and properties of the solid surface

*The Chemical Physics of Surfaces.* By S. Roy Morrison. Pp. xii+415. (Plenum: New York and London, 1977.) \$47.40.

SURFACE STUDIES constitute a substantial growth area in contemporary scientific research. During the past decade or so, a host of new techniques have provided insights into the structure and properties of the solid surface. These developments in turn have had a bearing on those technologically important processes where surface interactions are important—in solid-state devices, in oxidation, in corrosion and in catalysis.

In the past four years, several small monographs have appeared on the structure of the solid surface (Oudar, Blakely, Samorjai, Prutton); in those cases where reactions have been considered, however, they have been treated largely in terms of the atomistic nature of the surface. The present book attempts to re-dress the balance by emphasising the electronic band structure of the surface and the role of surface states in determining the interaction of the surface with a gaseous or liquid environment. The book is thus aimed primarily at those interested in surface chemistry and tends to deal more with semiconductors than with other types of solid.

The author expresses the hope that the book will also appeal to the mature surface physicists but it seems to me that it is too specialised and that it lacks more figures of the type given in Figures 5.1 and 5.2, where the lobes of orbitals and so on are of particular assistance to the non-chemist, and a few more energy diagrams illustrating the electron or hole switching mechanisms would clarify some of the verbal accounts of surface interactions.

Again, the references (almost 1,000) cover mainly the period from 1970 to 1975. The book is thus essentially a concentrated review of the more important papers published over a relatively short period. As such, it is indeed a tribute to the author's encyclopaedic knowledge and understanding of recent work involving general aspects of surface interactions, especially in relation to semiconductor surfaces. It does, however, carry with it certain limitations. For example, because of Dr Morrison's overwhelming interest in catalytic processes and in the chemical properties of ionic and semiconducting solids, his account of surface interactions gives only passing reference to faceting and little more to the role of surface defects.

One of the most exciting (and perhaps depressing) features of surface science is the rate at which new techniques are continuously emerging. Dr Morrison's book deals with papers up to the end of 1975 and quotes ultraviolet photoelectron spectroscopy (UPS) as "the most important tool of the surface spectroscopies in providing us with information on surface states and adsorbate bonding". Since then, high resolution energy loss spectroscopy (HRELS) at extremely small electron energies has appeared on the scene and has shown that it is possible to detect the vibrational characteristics of adsorbed atoms or molecules. It thus promises to provide more direct in-

formation than UPS about the location and bonding of surface species. No doubt in two years' time it will be superseded by yet another technique. This is not, of course, a criticism of Dr Morrison's monograph. His book is a treasury of information and an essential acquisition for any laboratory engaged in surface studies involving catalysis and solid-gas or solid-liquid interactions, especially of semiconducting solids.

David Tabor

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## Broad geophysical canvas

*Our Changing Planet.* By John Gribbin. Pp. 165. (Wildwood House: London, 1977.) £5.95.

THIS small book is intended to persuade laymen of the importance of geophysical research to Man's present needs, and also of its intrinsic interest. It ranges over a broad canvas, including the origins of the Solar System, oil, gas and metals, the possible relationship between sunspot activity, climate, biological evolution, and earthquakes, as well as the inevitable plate tectonics. This hotchpotch hangs together moderately well and avoids the doom-watch approach which often characterises such publications. The book is also well produced, with a pleasant appearance, a short layman's bibliography and an index. To complet the credit score, the number of printer's errors are few and these are amusing rather than annoying—larval plains on Mars, for example.

After commenting favourably on its readability, however, the credits become largely exhausted. The glib tone tends to be combined with an inadequate presentation, occasional misrepresentation and even straight errors. The importance of continental shelves as potential oil fields is clear enough without claiming that the Siberian Arctic continental shelf is larger than Africa, and the English Channel does not immediately jump to mind as exemplifying the characteristics of the shallow seas in which oil deposits are laid down. Greek ideas on the cause of earthquakes, dismissed as silly, still seem appropriate in particular conditions, such as those at Agadir. Some arguments are seriously neglected, such as the isotopic information for the origin of the metal brines of the Red Sea, and others seem almost topsy-turvy.

Earth expansionists use the 'im-

proved' fit of the continents on a smaller globe as part of their evidence in favour of expansion yet Gribbin sees the fit as a clear argument against. Although this reviewer's feelings about expansion make him side with Gribbin on this, the lack of evidence for expansion on the Moon, Mars, Venus, and so on, are the more telling arguments and are ignored.

It is also somewhat distressing to find, apparently, how provincial geologists are, compared with geophysicists. Apparently, geologists are concerned only with the status quo, and it is geophysicists who are concerned with the changing Earth. Although such cynical comments can be made only too easily, the most serious drawbacks for the lay reader, however, must be the lack of adequate illustrations and of clear exposition as to what, for example, plate tectonics is. If the initial picture had been presented more clearly, then one could almost, but not quite, forgive some explanations—for instance, how can North America override a spreading ridge? Simply because the Atlantic is spreading westward faster than the Pacific is spreading eastwards! In many cases, the glibness overtakes the information which is necessary, even for the lay reader. The Chandler Wobble, for example, is described as the pole dancing around, in stately fashion, relative to the stars, but the uninitiated will want to know the scale of this dance—is it confined to a barn, a ballroom or a continent?

I am sorry that I cannot recommend this book to anyone, which is not merely unfortunate but depressing. If John Gribbin had spent much longer on its preparation and confined himself to only two or three topics, it could have been excellent—but he didn't.

D. H. Tarling

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# obituary

## Sir Landsborough Thomson

ARTHUR LANDSBOROUGH THOMSON died on 9 June 1977 at the age of 86. With his death we have lost the last of that small group of men who, in the early years of this century, brought into being the organisation in this country for the national support of scientific research. A noted biologist, with a natural talent for administration, he combined in his own person the qualities required for translating scientific policy into administrative practice. It was, therefore, fortunate that circumstances brought him, at an early stage in his career, into the position of chief of staff at the headquarters of the then embryonic Medical Research Council.

Born in Edinburgh in 1890, Thomson spent his formative years in Aberdeen where his father was Regius Professor of Natural History. After graduating as MA in 1911, he elected for an academic career in zoology. In preparation for this, it was decided, in line with the spacious traditions of those days, that he should first broaden his education. So he went to study, first at Heidelberg, and then in Vienna from where he only just succeeded in escaping before the outbreak of the First World War.

Arrived back in Scotland, he immediately joined the Argyle and Sutherland Highlanders. With that distinguished regiment he went to France and saw hard service. It was then that his flair for administration first revealed itself. As a result he was recruited to the staff and ended the war, in the rank of Lieutenant-Colonel, as an Assistant Quartermaster-General at General Headquarters. He thus came to take a different view of his future. Returned to civilian life, he went to the Treasury. But he was only there for a short while. Sir Walter Fletcher, the then Secretary of the Medical Research Council, was looking for a man to build up the administrative side of his organisation; and Thomson was the obvious choice for the post.

When Thomson joined the MRC the entire staff at headquarters, apart from the Secretary, numbered no more than half-a-dozen persons. When he left, some forty years later, the figure was 130. Nevertheless, even then, the staff was remarkably small when set against the size of the Council's organisation and the scope of its activities. In part this was due to its having grown

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naturally rather than as a result of reforms imposed from outside. More especially, however, it was due to Thomson's wide range of capabilities. With his scientific background, and his continued personal involvement in research in ornithology, he was never in danger of mistaking means for ends and complicating administrative procedures accordingly. For him the first task was to identify the scientific requirements of any given problem and then devise measures for meeting these. Not that he ever fell into the opposite error of ignoring those basic principles and methods that are applicable to all administration, whatever its purpose. But he required that every case be approached on its merits, never on the basis of precedent. That was the atmosphere that permeated the whole office under his sway. As a result, agreements were reached with the minimum of fuss and, although prospective applicants might go away disappointed, they could seldom, if ever, feel they had been misunderstood.

But Thomson was far more than the head of the Council's machinery for supporting its purely research interests. Having a realistic appreciation of the social and political context in which the Council operated, and a shrewd eye for impending developments, he brought his own expertise to bear on the formation of policies for translating scientific knowledge into practice. An example is the part he played in creating the Public Health Laboratory Service. Originally conceived, in the mid 1930s, as a defence against bacterial

warfare, this quietly came into being on the outbreak of the Second World War. Such things do not happen by accident. They are the fruit of realistic vision and informed understanding by those responsible for planning the logistics of such an operation. In the event, the threat this particular service was devised to counter never materialised, but the network of laboratories it provided proved so useful in extending our control over the ordinary infections of civilian life that it was put on a permanent footing and eventually transferred, as a going concern, from the Council to the National Health Service. That Thomson should have been accepted without question as chairman of the expert board which directed such a service, is a sufficient indication of how he was regarded by the scientific community. When I want the MRC, I was exceptionally fortunate in having him as a colleague and mentor, for to work with him was a professional education in itself.

Yet all the time he had another life: that of a distinguished ornithologist. From his earliest years, he had taken an informed interest in wild life. Later, this developed into his classical studies on bird migration. But, at heart, he was always a field naturalist and, in this context, his knowledge was encyclopaedic. Consequently, he was, at one time or other, chairman of all the major organisations for ornithology in Great Britain and, in 1954, President of the XIth International Congress held in Basle. After his retirement from the MRC, his interests in this field could develop freely. He travelled widely, especially in the tropics. He took a major part in the enquiry into the Serengeti Park. From 1954 to 1960, he was President of the Zoological Society of London, from 1964 to 1969, Chairman of the Council for Nature, and from 1967 to 1969, Chairman of the Trustees of the British Museum (Natural History). And, with the help of his devoted wife, he edited his great *New Dictionary of Birds*.

In spite of these commitments, when he came to retire, Thomson readily accepted an invitation from the Medical Research Council to write an account of its development. Having been personally involved in this for most of the period which it covered, he was in a unique position to undertake this task. The outcome, *Half a Century of Medical Research*, is far more than an



institutional history. In it he sets out, with characteristic objectivity, the various developments in the British organisation of medical research and the administrative lessons to be learned from these. As such its relevance extends beyond the sphere of its immediate subject to a central problem of the scientific and technological age in which we are now living; namely, that of providing, not only for the development of scientific knowledge, but also for its integration into the machinery of government.

Like his old schoolfellow, Wilson Jameson, the architect of the National Health Service, Thomson understood that for any human institution to be a success, it must satisfy both of two fundamental requirements. It must be in conformity with the realities of that it seeks to organise. It must equally be in conformity with the deeply held sentiments of those who have to make it work. In his own time, Landsborough Thomson contributed his quota to realising these conditions and, thereby, to creating the institutions required if activities that depend on acts of individual judgment are to be integrated into the structure of social organisation. That was the deeper significance of his life's work and his essential legacy to future generations.

Harold Himsworth

## Meirion Thomas

MEIRION THOMAS who died in his eighty-third year on 5 April 1977 at Tywyn in his native Wales will be remembered not only for his academic and athletic prowess but also for sterling personal attributes.

Born in North Wales in 1894, his early academic career as a student of University College of North Wales was interrupted when he was commissioned in the South Wales Borderers in 1914 and later transferred to the Special Companies Division of the Royal Engineers. On demobilisation in 1919 he went to Cambridge (Trinity Hall) where, inspired by his contacts with Gowland Hopkins, F. F. Blackman and Muriel Onslow, among others, he developed interests in plant physiology and biochemistry. He joined the Botany Department in the Newcastle division of Durham University as a lecturer in 1924, became Reader in Plant Physiology in 1942, and in 1946 Professor and Head of the Department where he remained until retirement in 1961.

His book *Plant Physiology*, first published in 1935, brought him to the fore because of its new approach to the subject with its emphasis on the experimental study of plant metabolism. It was reprinted and revised periodically to a fifth edition in 1973, always re-

taining his aim of educating students in the development of ideas and investigation rather than simply presenting the present state of knowledge. His main research interests as covered by his own publications embraced various aspects of respiratory and acid metabolism with occasional ventures into other fields e.g. melanism, but the sum total of his contributions to science and life could only be assessed by perusal of the long list of students, colleagues and friends who would readily attest that whatever they have achieved, and some have achieved great distinction, they owe much to their contacts at some stage with him.

Meirion Thomas's eminence in science was marked by his election to the Royal Society in 1949. Two other distinctions which gave him great pleasure, and of which he was justifiably proud, were the award of the Charles Reed Barnes Honorary Life-Membership of the American Society of Plant Physiologists in 1963, and the Honorary Degree of D.Sc. of the University of Wales in 1964.

At Cambridge he won a soccer blue and played cricket, being elected to the Crusaders Club in 1923. He continued enthusiastic participation in both with Northern clubs for many years and remained actively interested in them throughout his life. A few years after retirement, his love of Wales, its language, and its cultures drew him to Tywyn, but not as a recluse. He maintained contacts with all his friends, continued to attend scientific meetings and enjoy a full life. He will be remembered with respect and great affection by all who knew him.

S. L. Ranson

## A. V. Nikolaev

ACADEMICIAN Anatolii Vasil'evich Nikolaev, a leading Soviet expert in geochemistry and the utilisation of natural resources died on February 13, 1977.

Nikolaev was born in 1902. He graduated from the University of Leningrad in 1924, and soon afterwards became involved in the problems of resource utilisation. From 1927 to 1931 he was head of the Pavlodar Salt Expedition of the Commission to Study Production Resources of the Academy of Sciences of the USSR, and from 1931 to 1935 he headed a similar, combined, expedition to the Kulunda area. Between 1934 and 1957 he worked at the Institute of General and Inorganic Chemistry of the Academy of Sciences in the USSR, and, simultaneously, held various teaching posts in Moscow, at the Polygraphic Institute and the Institute of Non-Ferrous Metals and Gold, becoming a Professor at the

latter Institute in 1946. In 1957 he moved to Novosibirsk, to become Director of the Institute of Inorganic Chemistry of the Siberian Branch of the Academy of Sciences of the USSR. The following year he was elected a Corresponding-Member of the Academy of Sciences and a Praesidium Member of the Siberian Branch. He became a full member of the Academy in 1966.

Nikolaev's main works deal with the physical and chemical analysis of salt systems, thermography and radiochemistry. His interests were wide and his published titles reflect this diversity, ranging from *The Kulunda Salt Lakes and means of desalinating them* and *The physico-chemical study of natural resources*, to *Protection against radioactivity*, and *A study of the processes of oxidation of complex compounds of divalent platinum*.

In 1947, he won the Vernadskii prize, and, in 1967, on the occasion of his 65th birthday, he was awarded the Order of Lenin.

Vera Rich

## C. W. F. McClare

COLIN W. F. MCCLARE, lecturer in biophysics at King's College London, died on 4 January 1977 at the age of 39.

He won an open Exhibition to Emmanuel College Cambridge from Felsted School in 1955 and read Natural Sciences, specialising in chemistry. His interest in applying chemical concepts to biological problems was already apparent in his PhD project on free radicals in biology and was developed further when as a Beit Fellow he studied energy transfer in nucleic acids.

After his appointment at King's College in 1963, he turned his attention to fundamental problems in bioenergetics, especially muscle contraction. He stressed the importance of time scales in thermodynamics and proposed a muscle model employing stimulated resonant energy transfer. He strongly advocated that the secret of ATP lies in the molecular details of its hydrolysis and not simply in its overall free energy change.

Colin was a person of great enthusiasm and intensity of thought. His interests ranged from practical methods of analytical biochemistry to the social and philosophical implications of science. His search for excellence and his competitive spirit showed in his love of discussion and in his sporting activities. As a teacher, he inspired his students through an outstanding ability to make ideas come to life.

He is survived by his wife Gill. His stimulating and ever-helpful presence will be missed by his many friends.

W. R. Lieb  
I. Gonda

# newly on the market

These descriptions are prepared by the staff of *Nature* on the basis of material provided by manufacturers. The Reader Enquiry Coupon faces the inside back cover.

**Photoresist illuminators.** Oriel. The high efficiency 1,000 W Photoresist illuminators produce highly intense uniform, collimated beams rich in the longwave ultraviolet and shortwave visible, the sensitive wavelength region for most photoresists. The unique optical system provides very high collection efficiency and excellent uniformity with an extremely simple alignment procedure.

Circle No. 42 on Reader Enquiry Coupon

**Tube rotator mixer.** Gallenkamp. A new tube rotator/mixer suitable for both vertical mixing and horizontal tube rotation, the drum accepts two or four plates of clips. Rotation speed is variable between 6 and 60 rev. min<sup>-1</sup>, and a clutch on the drive motor allows the drum to be rotated by hand for easy loading and unloading. A built-in timer enables runs of up to 60 min to be terminated automatically. The overall dimensions are 430 × 240 × 340 mm high.

Circle No. 43 on Reader Enquiry Coupon

**pH meters.** Gallenkamp. Models 7 and 12 have large-scale meter readouts and are intended for routine and research operation respectively. Both read 0–14 ( $\pm 0.05$ ) pH and 1400–0–1400 ( $\pm 14$ ) mV. In addition, model 12 has an expanded scale which gives a full scale deflection for one pH unit at any value within the overall range or 100 mV range. Two additional, logarithmic, scales are provided for ion activity measurements. Models 109 and 113 have digital readouts. The former is a general purpose meter reading 0–14 ( $\pm 0.01$ ) pH and 1900–0–1900 mV. Model 113 also covers the full pH range and is accurate to  $\pm 0.005$  pH unit, for research applications. Additionally it reads 1799–0–1799 mV and has an expanded scale facility. For routine laboratory and field studies, a battery-operated pH meter (Model 13) is available. This reads 2–12 pH with a 2 pH overrange at both ends of the scale, and 300–0–300 mV. Models 7, 12 and 113 are suitable for Karl Fischer titrations and all the pH meters are supplied complete with combination electrode.

Circle No. 44 on Reader Enquiry Coupon

**Automatic tissue dehydrator.** Reichert-Jung UK. The EM Tissue Dehydrator completely relieves the technician of tissue preparation for electron microscopy. Also, the process is made completely safe—dehydration taking place in a fully enclosed chamber—and highly consistent results are ensured. Specimens are accommodated in up to 51 porous capsules and the dehydration cycle is achieved in 9 automatically controlled stages. Timing of each stage is adjustable to ensure conformity with existing procedures, the tissue being finally taken up to 95% alcohol. There is also provision for immersion in 100% alcohol and propylene oxide, under manual control but still within the enclosed specimen chamber. At each stage, alcohol is added at a predetermined rate, the volume being adjusted to keep overall volume constant throughout the process. The process may be started or stopped at any stage and an alarm, incorporating a variable delay of up to 1 h, indicates cycle completion. On starting, a 50 s delay ensures that the dehydrator is purged of all undesirable vapours before operation.

Circle No. 45 on Reader Enquiry Coupon



Reichert-Jung automatic tissue dehydrator.

**Fume cupboard.** Morgan & Grundy. Traditional fume cupboards contain many joints and corners. To overcome the problems this can cause, Morgan & Grundy have designed a new one piece fume cupboard liner. All corners are welded with a large radius and the base is an integral part of the fume cupboard, so there are no corners or joints. This new design is of stainless steel, making it ideal for radio isotope work. The baffle is also made of stainless steel and is easily removable.

Circle No. 46 on Reader Enquiry Coupon

**High stability optical benches.** Oriel. Many precision optical components mount on to the precision high stability optical benches. These utilise a unique dovetail rail with spring-loaded, wobble-free carriers. Low 'stiction' surfaces allow extremely smooth positioning of the carriers. With care these carriers can be positioned to a precision of 0.001 inch (25  $\mu$ m) without screw adjustments. Most important, when the carrier locking screw is tightened, the carrier does not shift.

Circle No. 47 on Reader Enquiry Coupon

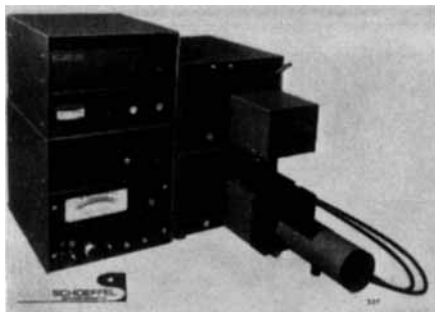
**Automatic blood oxygen dissociation analyser.** TCS. The continuous oxygen equilibrium curve of blood can be obtained with the TCS ABOD-analyser in 20 min. Less than one drop of whole blood or haemolysate and 20 min recording times are needed to plot a complete oxygen dissociation curve, and thus obtain the  $P_{50}$  value of haemoglobin. The curve is obtained by plotting the absorbance difference of haemoglobin as measured at two different wavelengths against the oxygen partial pressure ( $P_{O_2}$ ) as measured with a Clark type electrode.

Circle No. 48 on Reader Enquiry Coupon

**Precision saw.** Anshaw. The Model 7 precision saw from Anshaw Instruments features a choice of two types of quickly interchangeable cutting heads; wire or disk. The wire cutting head was designed to overcome the problem of damage introduced into crystal structures by normal cutting methods. It uses a 0.2 mm diameter, diamond impregnated, continuous wire 'blade' which can be run at speeds up to 10 m s<sup>-1</sup> and will cut fragile samples, as well as crystals, in practically all materials. The disk cutting head uses standard cutting disks of 100 mm diameter, either diamond or silicon carbide for all precision sawing, including metals, where the extreme sensitivity of the wire saw is not necessary. Various accessories are available, including a precision Goniometer for the orientation and cutting of crystals and also an attachment for automatically cutting circles and cylinders from crystals and hard, brittle materials. The saw has fully controllable cutting speeds and a cross-slide calibrated to 0.1 mm.

Circle No. 49 on Reader Enquiry Coupon





Schoeffel SF 770 and FS 970.

**Continuously variable wavelength absorption Monitor/Fluorescence Detector.** Schoeffel. By connecting the two units, the SF 770 and FS 970, in series and carefully monitoring the results, confirmation of the spectral characteristics of a compound can be accurately achieved. Determination of the most sensitive method of measurement (absorption or fluorescence) can be made. Extraction of valuable information regarding compounds not clearly separated from each other can be achieved when comparing separate recordings. Both units employ a high energy, low stray light monochromator and offer a selection of light sources (deuterium and tungsten). Both have solid state, miniaturised, modular design.

Circle No. 50 on Reader Enquiry Coupon

**Scale culture apparatus.** New Brunswick. The MultiGen, an alternative to the spinner flask procedure, is a 1 l or 2 l culture apparatus that can be converted for use with either microbial or suspension cultures. Fully integrated for the control of temperature, agitation, aeration, pH and dissolved O<sub>2</sub>, the MultiGen also provides facilities for the introduction of sparged air or a gas overlay and includes facilities for heating and cooling, turbine agitation or marine blade stirring. A magnetically coupled drive provides very gentle stirring to vigorous agitation and aeration while an electronic controller ensures reproducible speeds in a range up to 1,000 r.p.m. Precise temperature control from below ambient to 60 °C is achieved by a solid-state thermistor regulator; control accuracy is better than  $\pm 0.2$  °C. The MultiGen is equipped with its own air supply for aerobic studies. For anaerobic investigations, the pump can be switched off so that CO<sub>2</sub> or other gases may be introduced into the vessel.

Circle No. 51 on Reader Enquiry Coupon

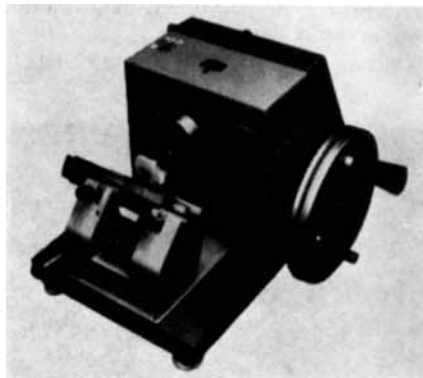
**Laboratory sample coater.** R. B. Electronics Ltd. A new precious metal coater designed for use in laboratories, the EMS 76 is a compact bench top instrument which diffuses a transparent layer of gold on to non-metallic laboratory samples to enable them to

be scanned by an electron microscope. The unit has solid state electronics and incorporates an efficient vacuum gauge which overcomes drift and contamination problems. The 'sputtering' method of coating eliminates the need for rotation or orbital motions. A typical coating time is 2 min for 300–400 Å of gold and the total cycle time is less than 5 min. Virtually any material can be coated, but it is ideal for use on samples of plant or animal tissue, fungus, synthetic fibres and plastics.

Circle No. 52 on Reader Enquiry Coupon

**Programmable electronic balance.** Sartorius. Model 3802 MP has a weighing range of 6,000 g with 0.1 g readability, operates on the principle of electromagnetic force compensation, and incorporates a microprocessor with a fixed programme to cater for specific problems of weighing. The pan (240 × 295 mm) is loaded and the weight read off in less than 2 s. The electronic memory of the balance stores each tare weight automatically after the tare sensor has been touched, while any other required random weights (e.g. standards for check-weighing or batch-weighing) can be similarly stored; only deviations from the standard will then be indicated. The 3802 MP can be used with data processors and control units such as printers, tape punchers, calculators, batch counters, recorders and remote readouts.

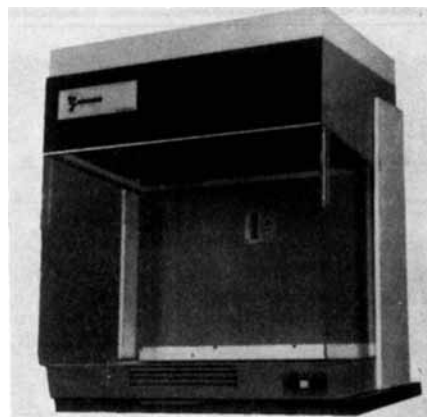
Circle No. 53 on Reader Enquiry Coupon



Reichert-Jung disposable knife holder.

**Disposable knife holder.** Reichert-Jung UK. A new disposable knife holder which fits AO, Reichert, Jung and virtually all other microtomes, overcomes the two most usual objections to the use of disposable blades—the questions of rigidity and adjustment of cutting angle. The holder is of the same size and general cross sectional profile as a standard non-disposable knife, allowing the normal angle adjustments on the microtome to be used. The disposable blade seating and gripping mechanism are so designed that the complete blade/holder assembly is extremely rigid enabling hard specimens to be sectioned without judder.

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Pathfinder downflow work station.

**Downflow work station.** Pathfinder. The new R133/6V range of bench mounted lamina downflow work stations are for use in medical research, diagnostic analysis, haematology and pathological laboratories, and the pharmaceutical, electronics and space industries. The R133/6V features easy operator access and top mounted, high efficiency filters, has air flow of 90 ft min<sup>-1</sup> (0.45 m s<sup>-1</sup>) and is fully recirculating with 10% front air make-up. The fully balanced, maintenance free fan system has been specially designed to ensure minimum vibration and a low sound level in the region of 60 dB (A scale) at 1 m. The largest unit in the range measures 1200 mm high by 1924 mm wide by 903 mm deep. The work surface is perforated stainless steel and side screens are of clear Perspex.

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**Conductivity meter.** Philips. The PW 9505 has a choice of 10 standard measuring ranges, from 0–3  $\mu\text{S cm}^{-1}$  up to 0–100 mS cm<sup>-1</sup> for which the new Philips plastic conductivity cell PW 9514/60 should be used. By using a specially developed screened cable supplied as standard with the instrument, together with certain Philips glass conductivity cells, the operator can cover the eight middle ranges. A large, easily read scale minimises reading errors, and gives high accuracy with 0.5% class meter. Facilities are provided for conductimetric titrations, reference measurements and ordinary resistance measurements. Reference temperature is normally 20 °C, but it can be extended to 25 °C with a minor modification.

The instrument provides a choice of manual or automatic temperature compensation. The manual mode covers the range from 0 to 50 °C. The automatic mode, carried out with Pt100/1 resistance thermometer or reference cell, covers the range from 0 to 120 °C.

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